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STUDIES ON THE TEMPERATURE OF INDIVIDUAL INSECTS, WITH SPECIAL REFERENCE TO THE HONEY BEE [1]

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INTRODUCTION

More observations have been made on the temperature of the bee colony than on the individual bee. Phillips and Demuth(*16*)[3] concluded that bees, essentially cold-blooded animals, were capable of regulating their temperature when in a colony by increasing the temperature of the colony when the air temperature went down and lowering the temperature of the colony when the air temperature went up. Since Phillips and Demuth have shown that the colony, which is composed of a large number of individuals, acts very much as a warm-blooded animal does, the temperature of the individual must be of great importance. If this were not true, it would be difficult to understand how the colony of individuals could control its temperatures. For this reason and because of the wide variation in the results obtained by previous workers this problem was chosen for study.

In a review of the literature on this subject it was found that previous workers had based the results of their observations on a few individuals. The results included in this paper are based on the readings of over 1,000 bees. The methods and results of the previous workers are given in the following review of the literature.

REVIEW OF LITERATURE

Hausmann (*10*) was the first to establish the temperature of an individual insect. He placed a Sphinx convolvuli together with a small thermometer in a glass receptacle, the air temperature of which was 17° R. (21.25° C.). After half an hour the temperature had risen to 19° R. (23.75° C.) and soon after fell to 17° R. Experiments with *Carabus hortensis* gave the same results.

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[2] The work on which this paper is based was done in the laboratories of the Division of Entomology of the University of Minnesota. To the members of the staff, and especially to Dr. R. N. Chapman, under whose immediate direction the study was conducted, acknowledgments are due for apparatus furnished and for constant helpful suggestions.

[3] Reference is made by number (italic) to "Literature cited," p. 286-287.

Davy (*4*) was the first to give the internal temperature of an insect. An incision was made in the body and the bulb of a very small mercurial thermometer was inserted. He obtained the following results:

	Air temperature.	Insect temperature.
	° C.	° C.
Blatta orientalis	28.3	23.9
Do	23.3	23.9
Gryllidae	16.7	22.5
Vespidae	23.9	24.4
Do	24.3	25.0
Lampyridae	22.8	23.0
Do	26.6	25.8

Nobili and Melloni (*15*) were the first to use electric methods in determining the temperature of insects. They used the electromotive force developed when the junction of wires of different metals of a common circuit are at different temperatures. They used bismuth and antimony wires. One of the thermocouples was in contact with the insect body; the other couple was free. They made a series of experiments on the temperature of larvae, pupae, and adult butterflies. Their conclusion was that the temperature of the insect was higher than that of the surrounding air.

Mussehl (*13*) ascertained that single bees became motionless at 5° R (6.25° C.) while they did not suffer from cold in the colony with the temperature of the hive at −1° R. (−1.25° C.).

Newport (*14*) used a thermometer of small caliber with a cylindrical bulb about one-half inch in length. The thermometer was placed beneath the insect and was as completely covered by the abdomen of the insect as possible. A second thermometer which had been carefully compared with the first was placed at the same level with and a short distance from the first one. The temperature of the insect was taken on the exterior and was always lower than that of the interior. He stated that the internal temperature was seldom if ever more than a degree and a half or at the most two degrees above the external temperature. The following are some of his results showing the difference between the air temperature and the external temperature of the insect:

	Temperature of air.	Body temperature.	Temperature difference.
	°F.	°F.	°F.
Bombus terrestris	66.9	73.4	6.5
Do	66.9	76.2	9.3
Do	66.9	73.4	6.5
Do	69.4	76.2	6.8
Do	68.0	77.5	9.5
Bombus lapidarius	68.0	71.5	3.5

At the time of taking the foregoing readings the insects were in an excited condition.

Dutrochet (7) used a thermocouple made by soldering an iron and copper wire together. He placed one couple in the body of the insect and the other couple in the body of a dead insect of the same species or in paper. He fastened several bees to a hive by means of a thread and took temperature readings while the bees were very active. One couple was placed 5 mm. deep in the body of the bee; the other couple was wrapped in paper to protect it from radiation. The air temperature was constant for four hours (19.2°C.). This experiment showed that the temperature of the insect was 0.18°C. lower than that of the surrounding air. The bees were next placed in a bell jar with the air dampened. They then had a temperature of 0.18°C. higher than the air temperature.

Experiments with *Bombus hortorum* were also performed. The thermocouple was not placed in the body but merely placed against it. The bee was then wrapped up in a piece of gauze which caused it to become very much excited. Under these conditions the temperature was 0.5°C. higher than that of the surrounding air. As the bee became quiet, its temperature was 0.03°C. lower than the air temperature. Dutrochet concluded that insects when active had a temperature higher than the surrounding air, when inactive a temperature that corresponded to that of the surrounding air.

Dzierzon (8) observed that bees became motionless at 5°R. (6.25°C.).

Dönhoff (5) placed 200 bees in a glass container the temperature of which was 22.5°C. In a short time the temperature had risen to 34.4°C. He also pressed an individual bee against a thermometer and found it to have a temperature of 15/16°C. higher than the air. He concluded that the difference in temperature between the bee and the surrounding air was greater when the air temperature was low and less when the air temperature was high.

Schönfeld (17) placed 100 bees in a water glass which was perfectly dry. A piece of old dried wax was attached to the cover of the glass in which there was a hole for the insertion of the thermometer. A small board was placed in the bottom of the glass on which the thermometer rested. The entire apparatus was placed on a cook stove. After 3 hours and 25 minutes the thermometer had risen from 10°R. (18.75°C.) to 31.5°R. (39.3°C.). All the bees, with the exception of 5, were found standing on the wax. One bee began to fan as the temperature reached 32.7°R. (40.8°C.) and at 36°R. (45.0°C.) 80 bees fell to the bottom of the glass and died. Sixteen bees were still living at 46°R. (57.5°C.), and when the cover was removed they flew out. The experiment was repeated and 2 bees withstood the temperature of 48.2°R. (60.2°C.) and flew away when the glass was opened.

Girard (9) carried on several experiments on insect temperature and found that the bumblebee had a lower temperature when in lack of honey. He concluded also that Hymenoptera had a higher temperature than the surrounding air but a significantly lower temperature than the Lepidoptera and Diptera.

Dönhoff (6) observed that bees soon died at −1.5°C. when placed in frozen ground.

Molin (12) found that bees became motionless at 5°R. (6.25°C.) and that at 7°R. (8.75°C.) they cleaned themselves and carried water. They left the hive and flew into the field at 12°R. (15°C.).

Koschewnikow (11) noted that bees kept at a temperature between 0° and 1°R. (0° to 1.25°C.) for 10½ hours lived. Other bees that were motionless for 30 hours at a temperature of −1°R. (−1.25°C.) also

survived. After the bees were kept at −2°R. (−2.5°C.) for 50 minutes they died.

In his work on maximum temperature he concluded that:

1. There was a range of 9° R. (11.25° C.) in the fatal temperature of bees.

2. Dryness and dampness had no effect on bees in a high temperature.

3. Workers and drones became very much excited when the temperature was above 30° R. (37.5° C.).

4. The highest minimum fatal temperature at which the workers died was 35° R. (43.75° C.), while the drones died at 30° R. (37.5° C.).

5. The highest temperature which the bees could withstand was 44° R. (55° C.).

6. The workers immediately upon hatching showed a sensitiveness to heat, none surviving more than 39° R. (48.75° C.).

7. Still younger bees with the chitin less hard but apparently well formed were in contrast to the ones mentioned above because they were more capable of withstanding a higher temperature. They died at 52° to 53° R. (65° C.). The explanation of this phenomenon appears to lie in the fact that their bodies contained and evaporated considerable moisture and that they did not move.

8. Upon bringing the bees immediatley into high temperature Koschewnikow observed that the period which passes between the time of inserting the bees and their death became shorter with increases in temperature.

In the following review of the experiment no statement was made as to whether or not the figures given were the actual internal temperatures of the insects.

Period of exposure.	Temperature to which bees were exposed.	Temperature of bees at death.
	°R.	°R.
2 minutes	44	45
2 minutes 10 seconds	45	46
2 minutes 5 seconds	46	46.75
2 minutes 15 seconds	47	48
1 minute 30 seconds	54	55
1 minute 10 seconds	55	55.5
1 minute 10 seconds	55.5	56
1 minute 10 seconds	56	56.25
45 seconds	57	57.5

Bachmetjew (1) performed several experiments on the temperature of insects, especially Lepidoptera. He used a thermocouple made by soldering steel and manganese wires together. Both wires were connected to a galvanometer, one directly and the second after it had passed through a commutator. The wires were insulated by passing them through small glass tubes. Butterflies were placed on the couple, and from many experiments Bachmetjew concluded that:

When the body temperature reached 39° C. the insect became very active and died at 46 to 47° C.

When the air temperature was lowered the body temperature of the insect was lowered to approximately −15° C.; there was then a rebound

in the body temperature from about −15° to about −1°. When the temperature of the body began to fall the insect died when the low point (−15° C.) was reached the second time.

Bachmetjew (*2*) made a general study of insect temperature in which he covered the work previously done on this subject.

Brunnich (*3*) made a study of the temperature of the bee body and the bee brood. He used a thermocouple made by soldering a copper and platinum wire together, forming the warm junction of the couple. To the free end of the platinum wire, which was only a few centimeters in length, he soldered another copper wire. This second union acted as the cold junction of the couple. He used the room temperature for his cold junction and made no allowance for changes in the temperature of the room. He also used a telescope reading galvanometer which he stated was not sensitive enough, since 15 seconds were required before the maximum reading was reached. The bees were greatly weakened by piercing with this rough thermocouple and soon died. He found that the body temperature of adult workers went as high as 39.6° C., while that of the drones went as high as 48.4°. The results, however, gave no indication of uniformity, because some individuals gave high temperatures while others, under the same conditions, gave low temperatures.

Since platinum is a good conductor of heat, there is great danger in having the warm and cold junction separated by a piece of wire only a few centimeters in length. In piercing the bee the temperature of its body or the handling of the wires, if held near the second couple, is likely to increase the temperature of that couple, thereby introducing an error in the readings. The increasing of the temperature of the second couple was undoubtedly the cause of the wide range in the body temperature cited by Brunnich in his paper.

METHODS

The piercing of the bees in the following experiment was done with a thermocouple (Pl. I, A), made by soldering No. 20 double cotton covered copper and constantin (a copper-nickel alloy) wire together. The wires were tapered to a fine point before soldering by inserting the ends in concentrated nitric acid and slowly withdrawing them. This process was repeated until the wires obtained the desired points. The tapered ends were then soldered together and the surplus solder filed off. A piece of cork was inserted between the two wires near their junction to strengthen the couple and aid in the handling of it.

The readings were made with the aid of a pyrovolter (Pl. I, C) and galvanometer (Pl. I, D), and the bee was pierced with a thermocouple of which the cold junction (Pl. I, B) was placed in a thermos bottle filled with ice and water to keep it at 0° C. A Northrup pyrovolter which has scales graduated into millivolts and degrees centigrade and a Leeds-Northrup outside galvanometer to aid in the setting of the zero point were used. By having one junction of the thermocouple in ice and water the resulting reading on the pyrovolter was the actual temperature of the bee in degrees centigrade.

One of the rooms in a cold-storage plant was used for the low temperature of 2.5°, 5.5°, 8°, and 9° C. For "room temperature," readings were made in the laboratory. Temperature readings for 27°, 30.5°, 35°, 39.5°, 43.5°, 52°, and 58° C. were made in a temperature box in

which the heat was regulated by a series of lights. The temperature box was made by placing insulite on the outside of the box 34 by 35 by 52 inches, the inside of which was lined with asbestos paper. There are two ventilating holes, one in the side near the bottom, the other in the top of the box.

At all temperatures the bees were allowed at least 10 minutes to become adapted to the surrounding air before the first of the group was pierced. When readings were made at 52° and 58° C. it was found that bees exposed to this high temperature for about 30 minutes were dead. The

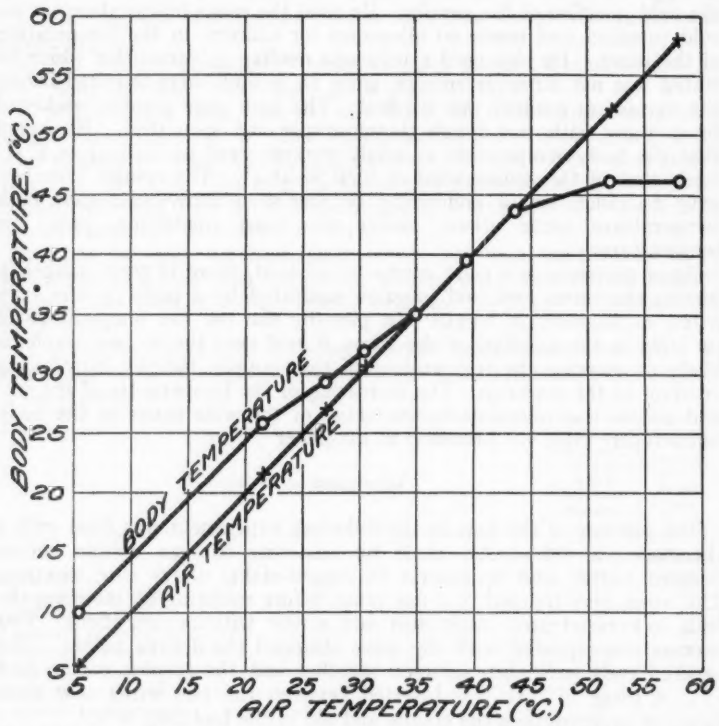

FIG. 1.—Graph showing the relation of body temperatures of Italian bees with the surrounding air. The bees which were taken from one hive were under winter conditions.

bees were placed in the temperature box at this high temperature in groups varying from 5 to 8 bees per group and exposed to the temperature for 10 minutes before any were pierced. The groups were placed in the box while its temperature was about that of the room. The lights were then turned on, and after the temperature had reached 52° or 58° and remained constant for 10 minutes the temperature readings were made.

The data for the temperature curve (fig. 1) were obtained from Italian bees that were all taken from one hive and in winter condition. On November 25 this hive along with about 90 others was placed in a

room in a cold-storage plant which maintained a temperature of about 7° to 7.5° C. throughout the experiment. A control was made by taking bees from different hives and placing them in groups, no two bees from the same hive in a group. A different group was used for each of the points on the curve.

Because of the popular belief that the Carniolian bees can withstand a lower temperature than the Italian bees it was thought advisable to make temperature readings on the Carniolians. Readings were taken at the temperatures of 2.5°, 5.5°, 8°, 9°, 30°, 35°, and 52° C. A group of 10 Carniolians was placed in rooms having temperatures of 2.5°, 5.5°, and 9° C. along with a group of 10 Italian bees. In piercing the bees an Italian was pierced first and then a Carniolian until all the bees were pierced. At 8°, 30°, 35°, and 52° the Carniolians were handled in the same manner as described for the Italians.

When taken from the hive the bees were placed in individual cages which allowed each bee the space of ¼ by ¼ by ⅝ inch. The cage was made from a block of wood ¾ by ¾ by 2 inches with a ¼ by ¼ inch groove in one surface. Cotton thread was used to wind around the block to prevent the escape of the bee. The thread also aided in piercing, as it permitted full view of the bee and eliminated the possibility of cross currents which might occur when using a wire screen to cover the groove. All piercing was done in the thorax, since there are no large air sacs in this region. It did not make any difference whether the dorsal or ventral surface was pierced, as is shown in Table I. By using a fine thermocouple there was no noticeable bad effect on the bee such as Brunnich (*3*) described in his paper. Several bees which were pierced, placed in a cage and fed, lived as long as bees not pierced and kept under the same conditions. Upon piercing these bees the second time, the same differences between the body temperature and that of the air were obtained as at the first piercing.

All thermocouples used in the experiment were carefully compared with the mercurial thermometer used by taking the readings of the temperature of the air before any of the bees were pierced. The following is an example of the results obtained in such comparison:

Air temperature (mercurial thermometer) 24° C.

Air temperature (thermocouple) 23.5°, 24°, 24°, 24°, 24°, 24°, 23.5°, 24°, 24°, 24° C.

TABLE 1.—*Body temperatures of bees at air temperature 5.5° C.*

Bee No.	Body temperature.	Surface pierced.	Condition of bee.	Bee No.	Body temperature.	Surface pierced.	Condition of bee.
	°C.				°C.		
1	11	Ventral	Active.	51	10	Ventral	Inactive.
2	11	Dorsal	Do.	52	9	do	Do.
3	12	do	Do.	53	9	Dorsal	Do.
4	11	do	Do.	54	9	do	Do.
5	10.5	do	Do.	55	9.5	do	Do.
6	11	do	Inactive.	56	10	do	Do.
7	10	Ventral	Do.	57	10.5	do	Do.
8	12	Dorsal	Do.	58	10	do	Do.
9	11	Ventral	Do.	59	10.5	do	Do.
10	11	do	Do.	60	10	do	Do.
11	11.5	Dorsal	Do.	61	10.5	do	Active.
12	14	do	Do.	62	13.5	do	Do.
13	12	Ventral	Do.	63	10.5	do	Do.
14	11	Dorsal	Do.	64	10	Ventral	Do.
15	11	Ventral	Do.	65	9	Dorsal	Do.
16	10.5	do	Active.	66	9.5	Ventral	Do.
17	11	Dorsal	Do.	67	9.5	do	Inactive.
18	11	do	Do.	68	10	do	Do.
19	10.5	do	Do.	69	9	Dorsal	Do.
20	10.5	Ventral	Do.	70	9.5	Ventral	Active.
21	10	Dorsal	Do.	71	13.5	do	Do.
22	9	do	Inactive.	72	11	do	Inactive.
23	9.5	do	Do.	73	10	do	Do.
24	9.5	Ventral	Do.	74	10	Dorsal	Do.
25	10	Dorsal	Do.	75	9	do	Do.
26	10	Ventral	Do.	76	10	do	Active.
27	10	Dorsal	Do.	77	10	do	Do.
28	10	Ventral	Do.	78	9	do	Do.
29	11	Dorsal	Do.	79	10	do	Do.
30	9.5	do	Do.	80	10	do	Do.
31	8.5	do	Active.	81	10	Ventral	Do.
32	9	Ventral	Do.	82	9.5	do	Inactive.
33	9	do	Do.	83	10.5	do	Do.
34	9.5	do	Do.	84	10.5	do	Do.
35	9.5	do	Do.	85	10	do	Do.
36	9	Dorsal	Do.	86	11.5	Dorsal	Do.
37	9	do	Do.	87	11	do	Do.
38	9	do	Inactive.	88	11	do	Do.
39	9.5	Ventral	Do.	89	10.5	Ventral	Do.
40	9	Dorsal	Do.	90	10.5	Dorsal	Do.
41	10	Ventral	Do.	91	9.5	do	Active.
42	9	do	Do.	92	10	Ventral	Do.
43	9.5	Dorsal	Do.	93	9	Dorsal	Do.
44	9.5	do	Do.	94	9.5	do	Do.
45	9.5	Ventral	Do.	95	9	do	Do.
46	10	do	Active.	96	9.5	Ventral	Do.
47	10.5	do	Do.	97	9.5	Dorsal	Inactive.
48	9.5	do	Do.	98	9	do	Do.
49	9	do	Do.	99	9.5	do	Do.
50	10.5	do	Do.	100	9.5	Ventral	Do.

Average body temperature 10.2° C. Difference between average body temperature and air temperature 4.7 °C.

TEMPERATURE OF THE ITALIAN WORKERS

A striking result noted in the tables was the small range in the differences between the maximum and minimum temperature readings at the different temperatures, as is shown in Table II. This is especially noticeable at the higher temperatures. In Table I there is a range of 5.5° C. between the maximum and minimum temperatures in the readings of 100 bees. We find in Table II a variation in the differences from 9° at 21.4° to 1.5° at 27° and 43.5° with the difference of 2.5° at the temperatures of 30.5°, 35°, 52°, and 58°.

TABLE II.—*Body temperatures of bees at various air temperatures*

Air temperature.	Average body temperature.	Difference between body and air temperature.	Maximum body temperature.	Minimum body temperature.	Number of bees pierced.
5.5	10.2	4.7	14	8.5	100
21.4	25.8	4.4	31	22.0	100
27.0	29.1	2.1	30	28.5	54
30.5	32.0	1.5	34	31.5	100
35.0	35.1	.1	37	34.5	100
39.5	39.5	.0	42	38.0	100
43.5	43.6	.1	44	42.5	100
52.0	46.0	−6.0	48	45.5	100
58.0	46.4	−11.6	48	45.5	11

That bees are capable of regulating their temperature for a short period of time was shown by the fact that when bees were placed in the temperature box at 52° and 58° C. their body temperature did not at once correspond to that of the air, as it did from 35° to 44°, but was lower. After the bees were exposed to this temperature for about 25 minutes, their body temperature approached nearer that of the air temperature. After they were in the temperature box for 30 minutes, they were dead and had the same temperature as that of the air. A few bees were tried at a low temperature −10° to +21°, and it was noted that the temperature of the body fell with that of the environment.

By handling the bees in individual cages they were not excited before piercing. The space allotted to each bee was large enough to permit it some movement, but not large enough for it to make use of its wings. Throughout the entire experiment only two bees, No. 62 and 71, in Table I, were observed to be fanning. They were immediately pierced and found to give a reading of 8° C. above that of the room, while those not fanning had a temperature about 4.6° above the air temperature. The bees were pierced at a low temperature and in the individual cages, so that the heat produced by the fanning was conserved, thereby raising their own temperature.

When bees were exposed to the temperature of 5.5° C., they were taken into the room in groups of 15. A period of three minutes elapsed before the piercing of each successive bee to permit the thermocouple to return to room temperature. After the piercing of about the sixth bee the remainder of them became motionless but were easily revived by holding them in the hand for a few minutes. After a group of 10 bees was kept in the room for a period of two hours they were pierced, and they gave an average temperature of 2° above the room temperature. After the bees were kept at this low temperature for 48 hours they were dead and recorded the same temperature as the surrounding air. The bees may have died from either starvation or from cold.

When readings were taken at "room temperature" the work was done in the laboratory and the temperature varied from 19.5° to 24° C., with an average of 21.5°. The bees were brought into the laboratory in groups of 10. At all other temperatures, with the exceptions of 52° and 58°, the bees were used in groups of 25.

As the air temperature was increased, the difference between the body temperature of the bee and the temperature of the air decreased until at

35°, or brood-rearing temperature, the two corresponded. As the air temperature went up from 35° to 44° the temperature of the bee and that of the surrounding air were the same, as shown in Table II.

When readings were taken at 52° and 58° C. the time factor entered, as the bees were at the point of their fatal temperature (about 46° to 48°). A bee was placed in the temperature box for 20 minutes before piercing. The thermocouple was placed in the thorax and kept there for seven minutes, readings being recorded every minute, during which time all movement ceased. Seven minutes later, when the couple was again inserted in the thorax, the body temperature of the bee was identical with that of the surrounding air. For the 100 bees used at 52° there was an average body temperature of 46°. If left at this temperature for 30 minutes the bees died, which indicated that the fatal temperature was around 46° to 48°.

About 250 bees were placed in a half pint (250-cc.) bottle. A thermocouple inserted near the top of the bottle gave a reading of 32° C. The temperature of the air in the room was at 25°. When the thermocouple was inserted farther into the bottle, so that the bees clustered on the wires, the thermocouple registered 34°. Later, when the bees were quiet, the couple was reinserted and the temperature was at 29°. The bees were agitated and the temperature went up to 32°. Temperature readings were taken of individual bees, while the cluster was at 32°, and they gave an average temperature reading of 34°.

Ten of these bees placed in a 10-cc. vial gave a temperature reading of 32° C. When the bees were quiet, they had a temperature of 29°. Agitating the bees caused their temperature to rise slowly until it reached 32° and remained there as long as the bees were active. This demonstrated that the bees can effect the temperature of their environment. If the heat radiated by the bee can be conserved, the temperature of the environment will go up. This in turn will permit the bee to raise its own temperature correspondingly, as there is a constant relationship between the body temperature of the bee and that of the environment it is in.

As is shown by the temperature curve (fig. 1) the supply of oxygen was a large factor in the amount of radiation that was set up by the bees. If the oxygen is limited, as may be done by corking the bees up tight in a bottle, the bees become inactive and the temperature goes down. If, on the other hand, the cork is removed and fresh oxygen is admitted, the bees give an immediate response.

TEMPERATURE OF ITALIAN DRONES

A few temperature readings were made on the drones which were taken from the hive early in the morning before they had left the hive to fly. At room temperature, 23° C., the body temperature of the drones corresponded to that of the workers, while at 52° the drones, average temperature was 42.4° compared with 46° for the workers.

TEMPERATURE OF CARNIOLIAN WORKERS

In work on the Carniolians more emphasis was placed on the lower temperature than on the higher ones. In Table III we find that there is no difference between the Carniolian and Italian bees for the temperatures of 2.5°, 5.5°, and 9° C. A comparison of Tables II and III for the temperatures of 30°, 35°, and 52° gives the same results.

TABLE III.—*Comparison of body temperatures of Carniolian and Italian bees at various air temperatures*

Number of bees pierced.		Air temperature.	Average body temperature.		Difference between body and air temperatures.		Maximum body temperature.		Minimum body temperature.	
Carniolians.	Italians.		Carniolians.	Italians.	Carniolians.	Italians.	Carniolians.	Italians.	Carniolians.	Italians.
		°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.
10	10	2.5	4.9	5.1	2.4	2.6	7	6	4	4.5
8	8	5.5	8.5	8.5	3.0	3.0	9	9	8	8.0
.....	.....	8.0	13.2	.....	5.2	.....	16	.....	11.5	25.0
10	10	9.0	13.8	13.6	4.8	4.6	16.5	16.0	12.5	12.5
25	.....	30.0	32.1	.....	2.1	.....	33.0	.....	31.5	.....
25	.....	35.0	35.0	.....	0.0	.....	35.0	.....	34.5	.....
25	.....	52.0	46.3	.....	−5.7	.....	48.0	.....	45.5	.....

FREEZING POINT

A study of the freezing point and the phenomenon of supercooling of the bee was made. An ether bath and a potentiometer were used. The ether bath was more easily controlled than an ice bath, and the temperature dropped about 0.5° C. per minute. Care was taken not to move the bee or have it come in contact with anything, as the least amount of movement will bring about the freezing of the insect. If the insect is moved or shaken about the time the rebound from supercooling to the actual freezing point is to take place, the bee will freeze and the rebound is not evident. It was also found that if the temperature of the bee when nearing its freezing point went down slowly, the freezing would take place and there would be no supercooling with a rebound to the freezing point. However, if the temperature went down rapidly as it neared the freezing point, the bee was supercooled and the rebound occurred. Three individual bees were taken down to −2.3°, when there was a rebound to −0.8°, or the actual freezing point. Another bee was supercooled to −4.3°, when the rebound occurred, the temperature going up to −2°., the actual freezing point. This conforms with the results obtained by Bachmetjew. The variation in the results is probably due to the fact that individuals do not have the same freezing point.

SUMMARY

The large variation in the results obtained by the early workers was due to the differences in methods of performing their experiments. Many of the old workers used mercurial thermometers, or thermocouples, which were then just coming into use. Nobili and Melloni (*15*) were the first to ever use this method. Because of the wide variation and unrefinement of the methods used by the early workers and the results they obtained their work remains only of historical value.

Bachmetjew (*2*) has contributed considerably to the general work on insect temperature by bringing together and giving a summary of the older works. In addition to this, he has performed many original experiments on the temperature of individual insects.

Brunnich (*3*) is the most recent worker on the temperature of bees. The lack of uniformity in his results is undoubtedly due to the fact that

he used the room temperature for the cold junction of his thermocouple. This cold junction was not controlled at a constant temperature, but was subject to fluctuations due to changes in the temperature of the room and also to the heat conducted along the short platinum wire from the bee which was pierced and from the hand with which the thermocouple was held while piercing the bees and taking the readings. Thus fluctuations in readings which Brunnich attributed to fluctuations of the temperature of the bee were more probably due to the fluctuations in the temperature of the cold junctions.

The author obtained very uniform results by using refined thermocouple methods and by having the cold junction in ice and water, keeping it constantly at 0° C.

CONCLUSIONS

From the foregoing results the following conclusions were made:

The average body temperature of the bee is 4.7° C. above that of the surrounding air when the air temperature is 5.5° and coincides with the air temperature when that is between 35° and 44°.

At 52° C. or above the temperature of the bee's body is lower than that of the air if not exposed to the high temperature for a long period of time.

The maximum fatal temperature of bees is about 46° to 48° C., and the freezing point is about −1°.

There is no appreciable difference between the body temperature of the Carniolian and Italian bees.

Bees are not wholly subject to the temperature of their environment, but are capable within certain limits of regulating their body temperature.

The ability of a colony to regulate its temperature is undoubtedly due to the ability of the individual to regulate its body temperature plus the ability to regulate and conserve the heat produced.

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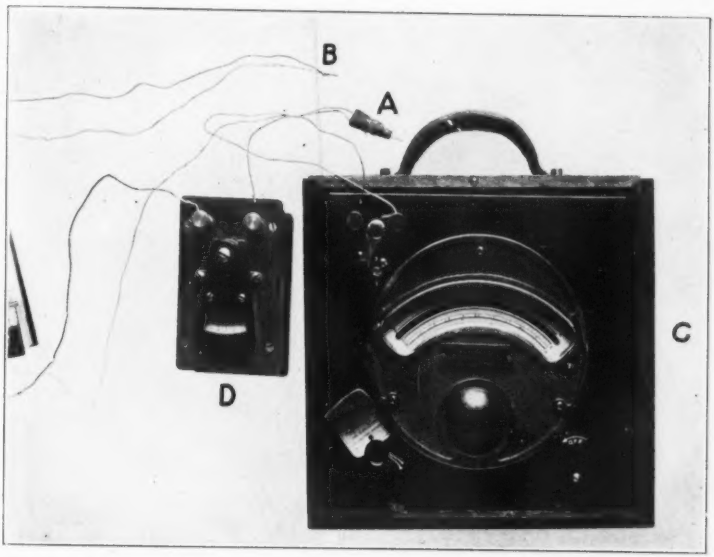
PLATE 1

A.—Warm junction of the thermocouple.
B.—Cold junction of the thermocouple.
C.—Pyrovolter.
D.—Galvanometer.


B
A
C
D

A STUDY OF THE EFFECT OF CHANGING THE ABSOLUTE REACTION OF SOILS UPON THEIR AZOTOBACTER CONTENT [1]

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INTRODUCTION

In studying the apparent correlation between the absolute reaction of soils and the presence of Azotobacter it occurred to the writer that if such a correlation existed one ought to be able, by varying the reaction, to control the Azotobacter content of any soil. To test the correctness of this view three lines of investigation were suggested.

(1) If two soils, one with a hydrogen-ion concentration favorable to the growth of Azotobacter, the other unfavorable, are mixed in varying proportions the hydrogen-ion concentration of some mixtures should be favorable and others unfavorable to the growth or existence of this group of organisms. All mixtures giving a hydrogen-ion concentration less than the maximum tolerated by Azotobacter should, upon subsequent analyses, show their presence. On the other hand, all mixtures giving a hydrogen-ion concentration greater than the maximum should not show the presence of Azotobacter, provided a sufficient incubation period elapsed to bring about their destruction.

(2) If the absence of Azotobacter in any soil is due to its high hydrogen-ion concentration, one should be able to decrease the acidity by the addition of increasing quantities of nontoxic basic materials, to a point where, once introduced, Azotobacter would survive.

(3) If a high hydrogen-ion concentration is inimical to the presence of Azotobacter one should be able, by the addition of acid, to increase the acidity of any soil containing Azotobacter to a point beyond the maximum tolerated by these organisms and thereby cause their disappearance.

The purpose of this paper is to report a few typical examples of a large number of experiments that have been carried out along these lines.

METHODS

The methods employed in the experiments here reported were similar to those previously reported.[2] Tests for Azotobacter were made by inoculating a mannite cultural solution with 10 per cent of soil, or the equivalent, as a suspension. Incubation was at room temperature. At frequent intervals during incubation the cultures were examined macroscopically to ascertain whether or not a film characteristic of Azotobacter was present. If the film was not typical and there were any indications of Azotobacter being present a microscopic examination was made. If the evidence thus obtained showed the presence of Azotobacter, it has been indicated by a plus sign in the following tables. A minus sign

[1] Accepted for publication June 29, 1922. Contribution No. 48, Department of Bacteriology, Kansas Agricultural Experiment Station.

[2] GAINEY, P. L. SOIL REACTION AND THE GROWTH OF AZOTOBACTER. *In* Jour. Agr. Research, v. 14, p. 265–271. 1918.

signifies the absence of Azotobacter. Where a question mark has been inserted it was impossible to tell whether Azotobacter were present.

The hydrogen-ion concentration determinations were made either colorimetrically after Gillespies method,[3] or electrometrically upon suspension of the soil. For the latter purpose a Leeds and Northrup potentiometer outfit was used in connection with saturated K Cl—calomel and hydrogen electrodes similar to that described by Hildebrand.[4] In general the two methods agreed very closely.

The mixtures of soils, or soils with various additions, were placed in 500-cc. wide-mouthed bottles plugged with cotton, and the moisture content was brought to a favorable point. Incubation was at room temperature. At frequent intervals the moisture lost through evaporation was restored. A more detailed description of the methods employed will be presented when the data are published in full.

RESULTS

MIXING SOILS

In the two experiments reported in Tables I and II, two soils (A and E), containing a vigorous Azotobacter flora, and one soil (B), in which Azotobacter have never been found, were studied. Soil A is approximately neutral, P_H 6.94. Soil E contains a high percentage of limestone and is alkaline, P_H 7.73. Soil B is strongly acid, P_H 3.65. Soils A and B would, under normal conditions, be expected to have approximately the same reaction, since the two are from similar locations and were taken only a few yards apart. However, the soil where B was taken was planted to pine trees a number of years ago and the high acidity is undoubtedly due to the decomposition of the highly acid pine needles. Extensive use has been made of this soil because it is the only strongly acid soil yet located in this immediate vicinity.

Previously reported experiments,[5] indicated that the maximum hydrogen-ion concentration tolerated by Azotobacter in soils is near 1×10^{-6}, or P_H 6.0. If P_H 6.0 represents the maximum acidity tolerated by this group of organisms, then all mixtures of soils A and B or E and B less acid than P_H 6.0 should, upon subsequent analyses, show Azotobacter, while all mixtures in which the acidity was very much greater than P_H 6.0 should not show their presence. It is probable that Azotobacter can exist for some time in a hydrogen-ion concentration that would not permit growth. This being true, some of the less acid samples in which Azotobacter can not grow might be able to initiate the growth of Azotobacter in a cultural solution. It would be expected that the higher the hydrogen-ion concentration the more rapidly the Azotobacter would be destroyed. From the relative reactions of the three soils it would also be expected that the quantity of soil A necessary to add to soil B in order to reduce the acidity of the mixture to P_H 6.0 would be larger than would be required in soil E. A glance at the second, third, and fourth columns of Tables I and II will show that in mixtures of soils A and B the ratio of A to B required to give an acidity less than P_H 6.0 lies somewhat between 9 to 1 and 4 to 1. In mixtures of soils E and B the ratio of E to B required to give the same reaction is 1 to 3.

[3] GILLESPIE, L. J. THE REACTION OF SOIL AND MEASUREMENTS OF HYDROGEN-ION CONCENTRATION. *In* Jour. Wash. Acad. Sci., v. 6, p. 7–16, 2 fig. 1916.

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[5] GAINEY, P. L. OP. CIT.

TABLE I.—*Effect of mixing an alkaline and an acid soil upon presence of Azotobacter. Mixtures of soils A and B* [a]

Sample No.	Grams of soil A.	Grams of soil B.	P_H	Azotobacter cultures					
				No $CaCO_3$ added to soil.			$CaCO_3$ added to soil.		
				Jan. 30.	May 22.	Dec. 11.	Jan. 30.	May 22.	Dec. 11.
1	300	0	7.02	+	+	+	+	+	+
2	300	0	6.86	+	+	+	+	+	+
3	270	30	6.42	+	+	+	+	+	+
4	270	30	6.32	+	+	+	+	+	+
5	240	60	5.76	+	+	+	+	+	+
6	240	60	5.82	+	+	+	+	+	+
7	150	150	4.60	+	+	−	+	+	+
8	150	150	4.61	+	−	−	+	+	+
9	60	240	4.11	+	−	−	+	+	+
10	60	240	4.09	+	−	−	+	+	+
11	30	270	3.79	+	−	−	+	+	+
12	30	270	3.90	+	−	−	+	+	+
13	15	285	3.77	+	−	−	+	+	+
14	15	285	3.75	+	−	−	+	+	+
15	3	297	3.75	+	−	−	+	+	+
16	3	297	3.68	+	−	−	+	+	+
17	1	299	3.66	+	−	−	+	+	+
18	1	299	3.65	+	−	−	+	+	+
19	0	300	3.68	−	−	−	−	−	−
20	0	300	3.62	−	−	−	−	−	−

[a] + = Presence of Azotobacter
− = Absence of Azotobacter
Experiment set up Jan. 30. Moisture content optimum. Acidity determined electrometrically.

TABLE II.—*Effect of mixing an alkaline and an acid soil upon presence of Azotobacter. Mixtures of soils E and B* [a]

Sample No.	Grams of soil E.	Grams of soil B.	P_H	Azotobacter cultures		
				May 1.	May 18.	June 21.
1	200	0	7.73	+	+	+
2	199	1	7.64	+	+	+
3	195	5	7.67	+	+	+
4	190	10	7.67	+	+	+
5	175	25	7.56	+	+	+
6	150	50	7.61	+	+	+
7	125	75	7.52	+	+	+
8	100	100	7.37	+	+	+
9	75	125	7.06	+	+	+
10	50	150	6.02	+	+	+
11	25	175	4.87	+	+	−
12	10	190	3.99	+	?	−
13	5	195	3.94	+	+	−
14	1	199	3.74	−	−	−
15	0	200	3.64	−	−	−

[a] ? = Azotobacter indefinite.
+ = Presence of Azotobacter.
− = Absence of Azotobacter.
Experiment set up May 1. Moisture content optimum. Acidity determined electrometrically.

A further examination of the two tables will show that, when cultured immediately after mixing, all mixtures of A and B and E and B, except 1 part of E to 199 parts of B, contained Azotobacter. Subsequent cultures, made after varying periods of incubation, show a gradual disappearance of Azotobacter in the more acid mixtures until only those samples contain them in which the acidity approaches very close to or is less than P_H 6.0. Numerous experiments have shown that the more acid a soil is the quicker will Azotobacter disappear therefrom when introduced, and that they can exist for some time in soils only slightly more acid than P_H 6.0. All the evidence accumulated so far, however, indicates that they can not remain indefinitely in a soil very much more acid than P_H 6.0.

ADDITION OF CALCIUM CARBONATE

In these experiments the aim was to find out whether lowering the hydrogen-ion concentration of a soil more acid than P_H 6.0, and not containing Azotobacter, would render the soil capable of supporting such a flora when introduced. The method followed was to add increasing quantities of various basic materials, particularly calcium carbonate, to acid soils containing no Azotobacter, inoculate the soil with Azotobacter, and after varying incubation periods culture to see whether or not the introduced organisms survived.

TABLE III.—*Effect of $CaCO_3$ upon the longevity of Azotobacter in an acid soil (B)*[a]

Sample No.	Percentage of $CaCO_3$.	Pounds $CaCO_3$ per acre.[b]	Azotobacter cultures.			
			Sept. 1.	Oct. 21	Jan. 4.	Feb. 18.
1	0.0	0	+	+	−	−
2	.1	3,000	+	+	−	−
3	.2	6,000	+	+	−	−
4	.3	9,000	+	+	−	−
5	.4	12,000	+	+	−	−
6	.5	15,000	+	+	−	−
7	.6	18,000	+	+	+	+
8	.7	21,000	+	+	+	+
9	.8	24,000	+	+	+	+
10	.9	27,000	+	+	+	+
11	1.0	30,000	+	+	+	+
12	1.1	33,000	+	+	+	+
13	1.2	36,000	+	+	+	+
14	1.3	39,000	+	+	+	+
15	1.4	42,000	+	+	+	+
16	1.5	45,000	+	+	+	+

[a] + = Presence of Azotobacter. − = Absence of Azotobacter. Experiment set up Aug. 20. Moisture content optimum.
[b] 3,000,000 pounds soil.

In Table III it will be observed that a minimum of 18,000 pounds per acre (3,000,000 pounds), or 0.6 per cent calcium carbonate, were required to render soil B capable of supporting an Azotobacter flora. Unfortunately the hydrogen-ion concentration of the samples of soil of these experiments was not determined.

TABLE IV.—*Effect of basic compounds upon longevity of Azotobacter in an acid soil (B)*[a]

Sample No.	Basic compounds added.	Pounds per acre.[b]	P_H	Azotobacter cultures.		
				Feb. 9.	Mar. 18.	May 7.
1	0.		4.3	−	−	−
2	0.		4.3	−	−	−
3	0.01 per cent $CaCO_3$	300	4.3	−	−	−
4	.05 per cent $CaCO_3$	1,500	4.4	−	−	−
5	.10 per cent $CaCO_3$	3,000	4.7	−	−	−
6	.50 per cent $CaCO_3$	15,000	5.3	+	−	−
7	1.00 per cent $CaCO_3$	30,000	6.5	+	+	+
8	2.50 per cent $CaCO_3$	75,000	7.0	+	+	+
9	1.01 per cent Na_2CO_3	300	4.3	−	−	−
10	.05 per cent Na_2CO_3	1,500	4.8	−	−	−
11	.10 per cent Na_2CO_3	3,000	5.2	−	−	−
12	.50 per cent Na_2CO_3	15,000	5.8	−	−	−
13	1.00 per cent Na_2CO_3	30,000	6.5	−	−	−
14	2.50 per cent Na_2CO_3	75,000	[c] 8.6	−	−	−
15	.01 per cent $Mg.CO_3$	300	4.3	−	−	−
16	.05 per cent $Mg.CO_3$	1,500	4.3	−	−	−
17	.10 per cent $Mg.CO_3$	3,000	4.3	−	−	−
18	.50 per cent $Mg.CO_3$	15,000	5.4	+	−	−
19	1.00 per cent $Mg.CO_3$	30,000	6.6	+	+	+
20	2.50 per cent $Mg.CO_3$	75,000	7.8	+	−	−

[a] + = Presence of Azotobacter. − = Absence of Azotobacter. Experiment set up Jan. 21, Moisture content optimum.
[b] 3,000,000 pounds soil.
[c] Greater than 8.6.

The data reported in Table IV were secured from the same soil, but the variations in the quantity of basic material added were much greater. Here again 15,000 pounds of calcium, magnesium, or sodium carbonate failed to reduce the acidity to P_H 6.0 or to make conditions favorable for the existence of Azotobacter. The P_H where 15,000 pounds of calcium carbonate were added was 5.3. Where 30,000 pounds, the next largest quantity, were added the P_H was 6.5 and the sample contained Azotobacter at all subsequent analyses.

Soil G with a reaction very close to P_H 5.8 (colorimetrically) was used in the experiments reported in Table V. The quantity of calcium, sodium, or magnesium carbonate necessary to reduce the acidity to P_H 6.0, or less, was not as great as was required by soil B. Two months after incubation started all samples except No. 12 (Table V) contained Azotobacter. In this particular case the high concentration of magnesium carbonate had apparently destroyed all organisms of this group. At the next analyses, seven weeks later, Azotobacter had disappeared from all samples more acid than P_H 6.0 and still remained in all those with a less high hydrogen-ion concentration, except when apparently destroyed by the high concentration of magnesium and sodium carbonate.

When excessive quantities of sodium and magnesium carbonate were added they apparently became toxic to the Azotobacter. The quantity necessary to produce such a condition was greater in soil G than in soil B. Lipman [6] has reported a toxic effect upon Azotobacter from both sodium and magnesium carbonate.

[6] LIPMAN, Charles B., and SHARP, L. T. TOXIC EFFECTS OF "ALKALI SALTS" IN SOILS ON SOIL BACTERIA. III. NITROGEN FIXATION. *In* Centbl. Bakt. [etc.], Abt. 2, Bd. 35, p. 647–655, 1 fig. 1912.
LIPMAN, Charles B., and BURGESS, Paul S. THE PROTECTIVE ACTION, AGAINST $MgCO_3$ OF $CaCO_3$ FOR A. CHROOCOCCUM. *In* Jour. Agr. Sci., v. 6, p. 484–498. 1914. Bibliographical footnotes.

TABLE V.—*Effect of basic compounds upon the longevity of Azotobacter in an acid soil (J)*[a]

Sample No.	Basic compounds added.	Pounds per acre.[b]	P_H	Azotobacter cultures.		
				Feb. 10.	Mar. 19.	May 8.
1	0.01 per cent $CaCO_3$	300	5.9	+	+	−
2	.05 per cent $CaCO_3$	1,500	6.0	+	+	−
3	.10 per cent $CaCO_3$	3,000	6.2	+	+	+
4	.50 per cent $CaCO_3$	15,000	7.4	+	+	+
5	1.00 per cent $CaCO_3$	30,000	7.4	+	+	+
6	2.50 per cent $CaCO_3$	75,000	7.4	+	+	+
7	.01 per cent Na_2CO_3	300	5.8	+	+	−
8	.05 per cent Na_2CO_3	1,500	6.0	+	+	+
9	.10 per cent Na_2CO_3	3,000	6.2	+	+	+
10	.50 per cent Na_2CO_3	15,000	7.6	+	+	+
11	1.00 per cent Na_2CO_3	30,000	[c]8.6	+	+	+
12	2.50 per cent Na_2CO_3	75,000	[c]8.6	+	−	−
13	.01 per cent $Mg.CO_3$	300	5.8	+	+	−
14	.05 per cent $Mg.CO_3$	1,500	6.0	+	+	+
15	.10 per cent $Mg.CO_3$	3,000	6.4	+	+	+
16	.50 per cent $Mg.CO_3$	15,000	7.6	+	+	+
17	1.00 per cent $Mg.CO_3$	30,000	8.0	+	+	+
18	2.50 per cent $Mg.CO_3$	75,000	8.6	+	+	−

[a] + = Presence of Azotobacter. − = Absence of Azotobacter. Experiment set up Jan. 21, Moisture content optimum. Acidity determined colorimetrically.
[b] 3,000,000 pounds soil.
[c] Greater than 8.6.

It is evident from the data presented in Tables III, IV, and V that the addition of calcium carbonate to an acid soil, not containing Azotobacter, if in sufficient quantity to reduce the hydrogen-ion concentration to approximately P_H 6.0 or lower, is all that is necessary to render the soil capable of supporting an Azotobacter flora. In no case, however, have Azotobacter been observed to appear in a soil so treated unless accompanied by inoculation either natural or artificial. In other words, Azotobacter are actually not present and must be introduced or the addition of calcium carbonate can have no effect upon nitrogen fixation by this group of organisms.

EFFECT OF ADDING ACID

In the experiments reported in Tables VI and VII, increasing quantities of various acids were added to soils A and C. Both these soils contained abundant Azotobacter. The reaction of soil A was very near P_H 7.0 and of soil C was near P_H 6.5.

Sulphuric, hydrochloric, acetic, and butyric acids were added to soil C reported in Table VI. Sulphuric and hydrochloric acid increased the hydrogen-ion concentration of the soil as the quantity of acid increased. As soon as the acidity passed much beyond P_H 6.0 Azotobacter were unable to survive prolonged incubation. When acetic or butyric acid was added it apparently had no effect upon the reaction, unless to reduce the hydrogen-ion concentration. This is contrary to the data presented in Table VII and is probably due to the time at which the hydrogen-ion concentration determinations were made. In Table VI the reaction was determined at the end of the period of incubation, while in Table VII the acidity determinations were made immediately

after being added or at the beginning of incubation. There are in soils organisms capable of utilizing many organic acids or their salts as food. This would cause the disappearance of the acid.

TABLE VI.—*Effect of adding acid to a soil containing Azotobacter (soil C)* [a]

Sample No.	Acid added to 200 gm. soil.	Pn.	Azotobacter cultures.	
			Mar. 2.	June 23.
1	0.2 cc. *N*/1 sulphuric	6.53	+	+
2	1.0 cc. *N*/1 sulphuric	6.73	+	+
3	5.0 cc. *N*/1 sulphuric	5.88	+	+
4	10.0 cc. *N*/1 sulphuric	5.29	+	(?)
5	20.0 cc. *N*/1 sulphuric	4.74	+	−
6	.2 cc. *N*/1 acetic	6.42	+	+
7	1.0 cc. *N*/1 acetic	6.61	+	+
8	5.0 cc. *N*/1 acetic	6.64	+	+
9	10.0 cc. *N*/1 acetic	6.74	+	(?)
10	20.0 cc. *N*/1 acetic	7.10	−	(?)
11	.2 cc. *N*/1 butyric	6.44	+	+
12	1.0 cc. *N*/1 butyric	6.58	+	+
13	5.0 cc. *N*/1 butyric	6.69	+	+
14	10.0 cc. *N*/1 butyric	6.70	−	+
15	20.0 cc. *N*/1 butyric	7.05	−	+
16	.2 cc. *N*/1 hydrochloric	6.41	+	+
17	1.0 cc. *N*/1 hydrochloric	6.51	+	+
18	5.0 cc. *N*/1 hydrochloric	5.58	+	−
19	10.0 cc. *N*/1 hydrochloric	5.51	+	−
20	20.0 cc. *N*/1 hydrochloric	4.51	+	−
21	0	6.50	+	+

[a] + = Presence of Azotobacter.
− = Absence of Azotobacter.
? = Azotobacter indefinite.
Experiment set up Feb. 16. Moisture content optimum.
Acidity determined electrometrically.

The increased growth of such organisms would bring about the accumulation of protein in their bodies which, upon decomposition, would result in the formation of more ammonia and hence a possible decrease in the hydrogen-ion concentration. The initial high acidity might result in either the partial or total destruction of the Azotobacter flora. If only a few organisms survived they might escape detection by the methods used. As soon, however, as the reaction again became favorable they would become abundant and be easily detected. This is probably what occurred in samples 14 and 15 of Table VI. On the other hand, if all the Azotobacter were killed they would never reappear unless inoculation took place, and subsequent analyses would fail to reveal their presence even though the reaction were favorable. The rapidity with which the Azotobacter are destroyed probably depends upon the degree of acidity, while the completeness with which they disappear from an acid soil depends upon the length of time the organisms are in contact with the acid condition. In the examples just given their complete destruction would depend upon the initial acidity and the period that elapsed before the hydrogen-ion concentration was again reduced below the maximum tolerated.

The data in Table VII show that with increasing quantities of organic as well as mineral acids the hydrogen-ion concentration increases. From

the reaction of samples 6 and 18 in Table VII Azotobacter would not be expected to be present. For reasons just discussed, it is probable that their presence in these samples is due to the fact that the initial hydrogen-ion concentration did not exist sufficiently long to destroy them.

TABLE VII.—*Effect of adding acid to soil containing Azotobacter (soil A)* [a]

Sample No.	Acid added to 200 gm. soil.	P_H	Azotobacter cultures.	
			Aug. 22.	Oct. 10.
1	0	6.81	+	+
2	10 cc. 2*N* sulphuric	2.52	−	−
3	20 cc. 2*N* sulphuric	2.26	−	−
4	50 cc. 2*N* sulphuric	2.01	−	−
5	50 cc. 2*N* sulphuric+10 gm. $CaCO_3$	1.83	−	−
6	10 cc. 2*N* lactic	5.70	+	+
7	20 cc. 2*N* lactic	4.50	−	−
8	50 cc. 2*N* lactic	4.06	−	−
9	50 cc. 2*N* lactic+10 gm. $CaCO_3$	7.03	+	+
10	10 cc. 2*N* lactic	6.46	+	+
11	20 cc. 2*N* lactic	5.09	+	+
12	50 cc. 2*N* lactic	3.73	−	−
13	50 cc. 2*N* lactic+10 gm. $CaCO_3$	6.00	+	−
14	10 cc. 2*N* formic	6.20	+	+
15	20 cc. 2*N* formic	4.09	−	−
16	50 cc. 2*N* formic	3.43	−	−
17	50 cc. 2*N* formic+10 gm. $CaCO_3$	7.78	+	−
18	10 cc. 2*N* citric	4.60	+	+
19	20 cc. 2*N* citric	2.62	−	−
20	50 cc. 2*N* citric	2.10	−	−
21	50 cc. 2*N* citric+10 gm. $CaCO_3$	2.55	−	−

[a] +=Presence of Azotobacter.
−=Absence of Azotobacter.
Experiment set up Aug. 6. Moisture content optimum.
Acidity determined electrometrically.

The data presented in Table VII indicate that if sufficient calcium carbonate is added to neutralize the added acid the Azotobacter will not be affected. However, large quantities of calcium carbonate are ineffective unless the quantity is sufficient to maintain a favorable reaction.

CONCLUSIONS

(1) If two soils, one more acid than P_H 6.0 and containing no Azotobacter and the other less acid than P_H 6.0 and containing Azotobacter, are mixed in varying proportions, incubated for some time, and cultured for Azotobacter, all mixtures giving an acidity less than P_H 6.0 will show the presence of Azotobacter, while all cultures very much more acid than P_H 6.0 will fail to show Azotobacter.

(2) If sufficient calcium carbonate is added to a soil more acid than P_H 6.0 and not containing Azotobacter to reduce the hydrogen-ion concentration to less than P_H 6.0, the soil will support Azotobacter.

(3) If sufficient acid is added to a soil less acid than P_H 6.0 and containing Azotobacter to increase the acidity to a point very much greater than P_H 6.0, Azotobacter will disappear from the soil, provided this acidity exists for sufficient time to complete their destruction.

OXIDATION OF SULPHUR BY MICROORGANISMS IN BLACK ALKALI SOILS [1]

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The accumulation of sodium carbonate in the soil (black alkali soil) brings about a condition in which the soil practically has to be abandoned as far as utilization for the growth of plants is concerned. Irrigation, whereby the carbonates are washed out from the soil, brings about a temporary and unsatisfactory relief. If the sodium carbonate could be converted into sodium sulphate (white alkali), much more satisfactory results could be ontained, since, as pointed out recently by Vinson and associates (*13*),[2] white alkali is readily and completely leached out from soil, while black alkali resists leaching. The use of sulphuric acid on alkali soil has been suggested by C. B. Lipman and Sharp (*6*); this acid was found to exert a favorable influence upon the soil by neutralizing the carbonate and improving the physical condition of the soil through flocculating the colloids. However, the sudden introduction of large quantities of acid into the soil may have an injurious influence upon the soil microflora. Elementary sulphur would prove, in this respect, of greater benefit, since, not only would the injury to the soil microflora be less but the addition of small quantities of sulphur even may stimulate bacterial activities.

J. G. Lipman (*7*) was the first to suggest the use of sulphur for alkali soil, in order that the acid formed from the oxidation of sulphur may reduce the alkalinity of the soil and transform the sodium carbonate into sodium sulphate. O'Gara (*9*), when applying sulphur and sulphuric acid to soils, observed that there was a reduction of the carbonate and an increase in the sulphate content of the soil and a decided increase in the crop yield. Hibbard (*3*) demonstrated that by adding sulphur to alkali soil the alkalinity is neutralized, this effect being of great value in the reclamation of alkali land. Rudolfs (*10*) found on adding elemental sulphur, at the rate of 1,000 pounds per acre, that the reaction of the soil was reduced from P_H between 9.6 and 9.8 to P_H 9.3; with 2,000 pounds per acre, to P_H 9.2; with 3,000, to P_H 8.9; and with 3,500, to P_H 8.2. However, if black alkali soil is first leached, the use of 3,000 pounds of sulphur per acre will change the reaction of the soil from P_H 9.2 to P_H 7.7.

Although some of the sulphur may be oxidized in the soil without the intervention of life, as pointed out by Kappen and Quensell (*5*) and others, it is primarily as a result of activities of certain microorganisms that the rapid oxidation of the elemental sulphur takes place in both

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[2] Reference is made by number (italic) to "Literature cited," p. 305.

acid and alkaline soils. Two organisms have been isolated, which are responsible for this process. These two organisms are different in nature, in both their morphological and physiological characters: (1) *Thiobacillus thiooxidans*, isolated at this station from sulphur-soil composts and described in detail elsewhere (*15*), was found to be very active in oxidizing sulphur practically quantitatively in acid soils; and (2) *Thiobacillus B* was isolated from alkaline soils on which the sulphur had been actively oxidized. The organism resembles in general morphological and physiological characteristics *T. thioparus*, studied by Nathanson (*8*) Beijerinck (*1*), and others, and occurs commonly in the soil. It grows readily in alkaline media having a reaction equivalent to P_H 9.8 and oxidizes readily thiosulphate with an abundant formation of elementary sulphur. A detailed description of methods for the study of this organism and its relation to the oxidation of sulphur in the soil are published elsewhere (*14*). No definite proof can, however, be given that *T. B* is absolutely pure and not contaminated with *T. thiooxidans* or a related strain.

In the experiment reported below, a black alkali soil from the University of California Ranch near Fresno, Calif., was used. Dr. Hoagland, of the University of California, who kindly supplied the soil, stated that "the physical condition of the soil was bad, the soil was extremely alkaline, and no ordinary growth of plants is supported." The reaction of the soil was about P_H 9.6 to 9.8.

The soil was placed in 100-gm. portions in tumblers and the proper amount of sulphur was added and thoroughly mixed with the soil. Fifty mgm. of sulphur in 100 gm. of soil is equivalent to 1,000 pounds of sulphur per 1 acre of soil (2,000,000 pounds per acre basis). The proper quantity of water was added and the tumblers were incubated at 25° to 28° C. in the dark.

The reaction of the soil was determined by shaking thoroughly 5 gm. of soil with 10 cc. of water, centrifuging, then determining the hydrogen-ion concentration (P_H value) colorimetrically and in some cases electrometrically. The sulphates were determined by shaking the soil with distilled water (*2*) in a shaking machine for 2 to 6 hours, then filtering clear and precipitating as barium sulphate. The carbonates and bicarbonates were determined by the method outlined by Schreiner and Failyer (*11*).

Tables I and II show the course of reaction resulting from the oxidation of sulphur in alkali soil and the chemical transformations that have taken place as a result of these changes.

TABLE I.—*Course of sulphur oxidation in alkali soil*

Period of incubation.	No sulphur added.	50 mgm. sulphur per 100 gm. soil.	100 mgm. sulphur per 100 gm. soil.	200 mgm. sulphur per 100 gm. soil.	500 mgm. sulphur per 100 gm. soil.	1,000 mgm. sulphur per 100 gm. soil.
	P_H	P_H	P_H	P_H	P_H	P_H
20 days	9.6	9.6	9.1	9.0	8.9	8.7
42 days	9.6	9.4	9.0	8.5	7.1	6.7
62 days	9.6	9.4	8.5	7.8	7.6	7.0
86 days	9.6	9.3	8.6	8.0	7.2	6.8
105 days	9.6	9.1	7.9	7.2	6.0	4.4
167 days	9.6	7.9	7.4	6.7	5.7	3.5

TABLE II.—*Transformation of a black alkali soil into a white alkali* [1]

	No sulphur added.		50 mgm. sulphur per 100 gm. soil.		100 mgm. sulphur per 100 gm. soil.		200 mgm. sulphur per 100 gm. soil.		500 mgm. sulphur per 100 gm. soil.		1,000 mgm. sulphur per 100 gm. soil.	
	Individual tumblers.	Average.	Individual tumblers.	Average.	Individual tumblers.	Average.	Individual tumblers.	Average.	Individual tumblers.	Average.	Individual tumblers.	Average.
P_H	9.6	9.6	7.8 8.0 7.9	7.9	7.6 7.1 7.4	7.4	6.4 7.0 6.6	6.7	5.5 5.5 6.2	5.7	2.8 3.0 4.6	3.5
Sulphates (milligrams of S in 100 gm. of soil)	26 26 29.5	27.2	77.6 72.4 77.5	75.8	95.5 95.5 93.6	94.9	158.0 172.8 162.4	164.4	229.3 225.5 188.6	211.1	636.3 436.7 270.0	447.7
Percentage of sulphur oxidized	.....	.....	.....	97.2	.....	67.7	.....	67.6	.....	36.8	.....	42.05
Carbonates (milligrams of CO_3 in 100 gm. of soil)	47.2 51.2 49.2	49.2	7.9 5.9 5.9	6.6	.....	0	.....	0	0	.....	0	0
Bicarbonates (milligrams of HCO_3 in 100 gm. of soil)	182 178 172	177.3	102 104 104	103.3	70 72 74	72.0	38 32 34	34.7	20 15 24	20.0	0 0 10	3.3

[1] Cultures incubated 167 days at 25° to 27° C.

The results brought out in Tables I and II point out definitely that not only is elemental sulphur oxidized readily to sulphuric acid, which results in the neutralization of the carbonates of the soil, but the reaction of the soil can be brought down to any desired point, depending entirely upon the quantity of sulphur added and the length of time during which the sulphur is allowed to be in contact with the soil.

The next experiment deals with the oxidation of sulphur by pure and crude cultures of the sulphur-oxidizing bacteria in alkaline soil under sterile conditions. The soil was placed in 100-gm. portions in 250 cc. Erlenmeyer flasks; 100, 200, and 500 mgm. portions of sulphur were added to the soil portions, which were then thoroughly mixed, then the optimum amount of water was added and the flasks were sterilized for 1½ hours at 15 pounds pressure. The flasks were then inoculated by means of a sterile pipette, with 2 drops of the vigorously growing cultures and incubated at 25° to 28° C. Alkaline soils in which active sulphur oxidation had taken place were used as the crude alkaline compost. The results are given in Table III.

TABLE III.—*Oxidation of sulphur in alkaline soil by different bacteria*

Quantity of sulphur used.	Type of culture.	Incubated 14 days.	Incubated 30 days.			Incubated 60 days.			
		P_H	P_H	Carbonates in 100 gm. of soil (mgm. of CO_3).	Bicarbonates in 100 gm. of soil (mgm. of HCO_3).	P_H	Carbonates in 100 gm. of soil (mgm. of CO_3).	Bicarbonates in 100 gm. of soil (mgm. of HCO_3).	Sulphates in 100 gm. of soil (mgm. of S).
None	Control	9.6	9.6	49.0	159	9.6	52.5	143	30.1
100 mgm.	Crude alkaline compost	7.7	7.5	0	50.0	7.7	0	43.5	115.5
200 mgm.	do.	7.4	6.3	0	31.0	5.6	0	8.5	201.0
500 mgm.	do.	7.0	4.9	0	15.0	4.7	0	6.5	254.8
100 mgm.	*Thiobacillus thiooxidans.*	9.2	8.8	8.0	142.0	8.6	0	86.0	109.4
200 mgm.	do.	8.6	9.0	14.7	127.0	7.5	0	53.0	145.3
500 mgm.	do.	8.6	9.0	5.8	118.0	7.4	0	45.0	160.7
100 mgm.	*Thiobacillus B.*	8.8	9.0	29.4	132.0	8.8	0	80.0	78.2
200 mgm.	do.	8.2	8.0	18.5	138.0	8.0	0	55.5	118.4
500 mgm.	do.	8.0	7.8	15.6	136.0	7.6	0	48.0	123.3

The crude culture proved to be most efficient in oxidizing sulphur in alkaline soil to such an extent that the reaction has been reduced, where sufficient sulphur has been used, from most alkaline to distinctly acid. This was accompanied by a complete disappearance of the carbonates, an almost complete disappearance of the bicarbonates, and an increase in the quantity of sulphates. With 100 mgm. of sulphur per 100 gm. of soil, equivalent to 1 ton of sulphur per acre (on the basis of the upper 6⅔ inches of soil), 85 per cent of the sulphur has been oxidized by the crude culture to sulphates within 60 days, and the reaction reduced from P_H 9.6 to P_H 7.7. When the quantity of sulphur was doubled, practically the same percentage of sulphur (85.5) was oxidized in 60 days and the P_H changed to 5.6 Where a large excess of sulphur was used, or 500 mgm. per 100 gm. of soil, only 44 per cent of the sulphur was oxidized to sulphate, and the reaction of the soil changed to P_H 4.7, which would already prove injurious to certain crops because of the excess acidity. The pure cultures did not prove quite as effective as the crude culture. This may be due to the fact that the oxidation of sulphur in alkaline soil is carried out not by one organism but by several organisms taking part in the process, as is shown by the experiments reported in Table IV, where a mixture of the two organisms is used. The acid soil used in these experiments is a Sassafras sandy loam, slightly acid in reaction. These experiments were carried out both under sterile and nonsterile conditions in tumblers.

TABLE IV.—*Oxidation of sulphur in acid and alkaline soils by pure and crude cultures of sulphur-oxidizing bacteria*

Soil type.	Sterilization of soil.[1]	Organism.	Incubated 21 days.	Incubated 48 days.
			P_H	P_H
Acid; 100 gm. of soil + 100 mgm. of sulphur.	+	Control	6.0	5.8
	−	do	5.2	4.4
	+	Crude, acid compost	5.7	3.8
	−	do	4.8	3.8
	+	Crude, alkaline compost	5.4	5.0
	−	do	5.4	4.0
	+	*Thiobacillus thiooxidans*	5.6	5.0
	−	do	5.0	3.8
	+	*Thiobacillus B*	5.8	5.4
	−	do	5.4	4.0
	+	*Thiobacillus thiooxidans* + *Thiobacillus B*	5.4	3.4
	−	do	4.8	3.6
Alkali; 100 gm. of soil + 200 mgm. of sulphur.	+	Control	9.6	9.2
	−	do	8.8	8.2
	+	Crude, acid compost	9.0	8.2
	−	do	9.0	8.4
	+	Crude, alkali compost	7.5	7.1
	−	do	7.6	7.3
	+	*Thiobacillus thiooxidans*	8.8	8.0
	−	do	8.2	8.0
	+	*Thiobacillus B*	8.2	7.8
	−	do	8.0	7.6
	+	*Thiobacillus thiooxidans* + *Thiobacillus B*	7.8	7.6
	−	do	7.6	7.4

[1] + indicates that the flasks containing soil and sulphur were sterilized, at 15 pounds pressure, for 1½ hours; − = soil unsterilized, in tumblers.

In the acid soil, oxidation of sulphur took place in the unsterilized containers whether inoculated or uninoculated, more so in the inoculated cultures, particularly where the *Thiobacillus thiooxidans* was introduced. This is due to the fact that the sulphur added, in nonsterile condition, probably has been inoculated previously from the laboratory air. However, when the soil and the sulphur are previously sterilized, oxidation takes place only to a very unappreciable extent, unless the proper sulphur-oxidizing organisms are introduced. In the alkaline soil, the most efficient results were obtained from inoculation with the crude alkaline culture, or alkaline soil, in which sulphur oxidation had taken place previously. The pure cultures, particularly the *Thiobacillus B* culture, which was almost inactive in the acid (sterilized) soil, proved more efficacious than the *T. thiooxidans;* but a mixture of the two cultures proved nearly as efficacious as the crude alkaline composts.

The fact that the change of the soil carbonates to sulphates would also favor plant growth, which is found to be so in actual field results, was demonstrated in these experiments by the increase in the number of bacteria developing in the soil. The soils used in the experiment reported in Tables I and II were at the end of the experiment air dried, then again moistened and, after 5 to 6 days, the bacterial numbers were determined by the ordinary plate method. The alkali soil, to which no sulphur had been added, contained 320,000 bacteria per gram of soil; where 50 mgm. of sulphur had been added per 100 gm. of soil and the reaction changed to P_H 7.9, there were 665,000 bacteria per gram; 100 mgm. of sulphur, reaction P_H 7.4, 875,000; 200 mgm. of sulphur, reaction P_H 6.7, 1,275,000; 500 mgm. of sulphur, reaction P_H 5.7, 3,650,000; 1,000 mgm. of sulphur, reaction P_H 3.5, no bacteria, only a few occasional fungi. In this case, the soil reaction has been made so acid as to completely kill off the bacteria. This danger, however, could hardly be expected in the field, because no such large quantities of sulphur (1 per cent) would ever be employed.

In the following experiment, the reaction of the soil was adjusted by the addition of sulphuric acid and sodium carbonate. The following amounts of acid and alkali were used to adjust the reaction of the soil:

	P_H.
Untreated soil	5.6
5 cc. *N/2* H_2SO_4 per 100 gm. of soil	5.3
0.5 cc. *M/2* Na_2CO_3 per 100 gm. of soil	5.8
1.0 cc. *M/2* Na_2CO_3 per 100 gm. of soil	6.0
3.0 cc. *M/2* Na_2CO_3 per 100 gm. of soil	6.2
7.0 cc. *M/2* Na_2CO_3 per 100 gm. of soil	6.4
20.0 cc. *M/2* Na_2CO_3 per 100 gm. of soil	8.4

One-gm. portions of powdered sulphur were added to each 100-gm. portion of soil in Erlenmeyer flasks; the flasks were well shaken, plugged with cotton, sterilized at 15 pounds pressure for 1½ hours, then inoculated with pure cultures of the organisms and incubated at 25°C. At the end of definite intervals of time the moisture content was brought up by the addition of sterile distilled water. The results are presented in Table V.

TABLE V.—*Oxidation of sulphur by pure cultures of bacteria at various reactions*

Type of culture.	P_H value.				Soluble sulphates (sulphur) in 100 gm. of soil after 50 days' incubation.
	Initial reaction of soil.	16 days after.	30 days after.	50 days after.	
					Mgm.
Control	5.2	5.2	5.3	5.4	19.2
	5.6	5.6	5.6	5.6	13.7
	5.8	5.8	5.8	5.7	11.0
	6.0	6.0	5.8	5.8	13.7
	6.2	6.2	6.2	6.2	13.7
	8.4	8.4	8.2	8.2	21.9
Thiobacillus thiooxidans	5.2	5.2	3.4	2.8	315.1
	5.6	5.4	3.0	2.8	314.6
	5.8	5.6	5.5	5.5	20.6(?)
	6.0	5.8	3.0	2.6	358.0
	6.2	5.4	2.8	2.8	380.0
	6.6	6.6	6.6	3.0	304.4
	8.4	8.4	8.2	8.0	27.4
Thiobacillus B	5.2	5.3	5.2	5.4	39.7
	5.6	5.3	5.2	5.2	20.6
	5.8	5.5	5.5	5.4	21.9
	6.0	6.0	5.5	5.5	15.2
	6.2	6.0	5.8	5.4	19.2
	6.6	6.6	6.6	6.4	24.7
	8.4	8.2	8.0	7.8	.....
T. thiooxidans+*T. B*	5.2	5.2	3.4	2.8	302.5
	5.6	3.1	2.8	2.6	287.7
	5.8	2.8	2.8	2.6	311.1
	6.0	2.8	2.6	2.6	308.3
	6.2	2.8	2.8	2.6	367.0
	6.6	3.1	2.8	2.8	274.0
	8.4	8.2	8.0	7.4	.....

Both the reaction and the sulphur content of the control indicate that no sulphur or only traces of it were oxidized under sterile conditions. However, in the presence of the sulphur-oxidizing bacteria, the oxidation of the sulphur took place very rapidly, with the transformation of large quantities of sulphur to sulphuric acid. When the reaction of the soil was acid, the oxidation of the sulphur in 50 days was carried on entirely by *Thiobacillus thiooxidans*. *Thiobacillus B* oxidized only very small quantities of sulphur; but, in the presence of both organisms, the speed of the reaction was hastened, as indicated by the measurements of the P_H values obtained in 16 days. This may be explained by either of two assumptions: (1) If we suppose a so-called "lag phase" in bacterial development, *Thiobacillus B* produces substances which appreciably shorten the "lag phase" of *T. thiooxidans;* (2) *Thiobacillus B* may oxidize the sulphur not directly to sulphates but to other compounds of sulphur, which are then rapidly oxidized to sulphates by *T. thiooxidans*. This is made clear in the study of the pure cultures of *Thiobacillus B*, with sodium thiosulphate as a source of energy, when a large part of the thiosulphate is transformed to persulphates, a part to elementery sulphur, and only a part to sulphates. This preparatory function of the *Thiobacillus B* may explain a number of hitherto unexplained difficulties observed in the study of sulphur oxidation by microorganisms in the soil. Of these we need mention only a few.

Nathanson (*8*), who first studied the Thiobacillus group, of which *Thiobacillus B* (not *T. thiooxidans*) is a representative, stated that thiosulphate is oxidized to sulphate and tetrathionate, with the production of free sulphur; however, on sodium sulphid agar plates, when free sulphur is formed abundantly in a nonbiological way, this sulphur is oxidized by the bacteria. The reaction proposed by Nathanson ($3Na_2S_2O_3 + 5O = Na_2S_4O_6 + 2Na_2SO_4$) could hardly be justified, particularly in view of the fact that the sulphur was supposed to be produced in a purely nonbiological way, by the interaction of the tetrathionate with the remaining thiosulphate, and was not supposed to play any part in the reaction. Beijerinck (*1*), who identified Nathanson's organism with his *T. thioparus*, then suggested the following reaction:

$$Na_2S_2O_3 + O = Na_2SO_4 + S.$$

But Jacobsen (*4*), who worked in Beijerinck's laboratory, found that the same organism oxidized elemental sulphur quantitatively to sulphate, which would again hardly justify Beijerinck's formula, if the organism was the same. Trautwein (*12*), however, obtained the separation of elemental sulphur from thiosulphate only in crude cultures of the organisms; but, in pure culture, no separation of elemental sulphur was found. At the same time a large part of the thiosulphate was transformed to sulphate. At first he thought the following reaction to be justified:

$$Na_2S_2O_3 + 4\ O + H_2O = Na_2SO_4 + H_2SO_4.$$

In view of the fact that the reaction of the medium did not turn acid and that a large quantity of persulphate was formed, he finally concluded that the reaction takes place as follows:

$$\text{I.}\quad 4Na_2S_2O_3 + 12\ O = 4Na_2S_2O_6.$$
$$\text{II.}\quad 3Na_2S_2O_3 + 5\ O = Na_2S_2O_6 + 2Na_2SO_4.$$

In the investigations carried on in this laboratory with pure cultures of *Thiobacillus B*, it was found that this organism transforms thiosulphate into persulphate, sulphate, and elemental sulphur, with only a limited production of acid (P_H changed in culture solution from 9.8 to 6.4 or 7.0). When *T. thiooxidans* is added to the culture solution, the elemental sulphur separated by the *Thiobatillus B* is rapidly oxidized to sulphuric acid and the final reaction may go down from P_H 9.8 to P_H 1.2 as a result of the action of the two organisms. The culture solution used in this case consists of 5 gm. sodium thiosulphate, 1 gm. sodium bicarbonate, 0.2 gm. dipotassium phosphate, 0.1 gm. magnesium chlorid, 0.1 gm. ammonium chlorid, 0.25 gm. calcium chlorid, and 1,000 cc. of tap water.

These discrepancies in the type of reaction that takes place when thiosulphate is acted upon by sulphur-oxidizing bacteria can be explained by the fact that different workers used different forms of a closely related group of organisms; in some cases the culture used possibly contained not one organism but a mixture of two or more organisms. *Thiobacillus B*, isolated in this laboratory and closely related, in its morphology and physiology, to *T. thioparus* and allied forms studied by Nathanson (*8*), Beijerinck (*1*), Trautwein (*12*), and others (*4*), was possibly contaminated in some cases by *T. thiooxidans*, which oxidizes actively elemental sulphur and is described by Waksman and Joffe (*15*). *T. thioparus* will act upon sulphur compounds under distinctly alkaline conditions, as in

culture media containing soluble carbonates or bicarbonates or black alkali soil; the elemental sulphur will be oxidized only comparatively slowly, but, in the presence of *T. thiooxidans* this oxidation will be rapidly hastened and the reaction quickly changed to any degree of acidity, depending merely on the quantity of sulphur used.

The possibility of the participation of other organisms oxidizing sulphur or sulphur compounds in the soil, or in any way influencing the process, in addition to the two mentioned, is not excluded. Neither is the possibility excluded that the oxidation of sulphur in alkaline soil is carried on chiefly by a strain of *Thiobacillus thiooxidans* which has adapted itself to alkaline conditions. While *T. thiooxidans* grows vigorously on artificial media and does not deteriorate with age, *Thiobacillus B* rapidly deteriorates under cultural conditions and the very slow oxidation of sulphur by this organism, particularly in the latter part of the work, may be due to this phenomenon.

SUMMARY

(1) The use of sulphur in the presence of the proper sulphur-oxidizing bacteria will result in the transformation of black alkali soil to white alkali soil.

(2) The final reaction of the soil depends on the quantity of sulphur used and the length of time which the sulphur is allowed to be in contact with the soil.

(3) The oxidation of sulphur in black alkali soil is probably carried on by the agency of more than one sulphur-oxidizing bacterium.

(4) In the presence of two bacteria, one of which can act upon sulphur under distinctly alkaline conditions while the other rapidly oxidizes sulphur under acid conditions, the speed of the reaction is greatly hastened.

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PEACH ROSETTE, AN INFECTIOUS MOSAIC[1]

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HISTORY OF THE DISEASE

According to Smith[2] peach rosette was first noted in Georgia in 1881. By 1891 this disease had been reported from 22 counties in Georgia, from South Carolina, and from Kansas. Rosette was also reported as occurring on plums in Georgia and Kansas, and on almonds in Kansas.

Since 1903, reports of the presence of rosette have come to the Plant Disease Survey, of the United States Department of Agriculture, from 19 counties in Georgia, 1 county in Alabama, 5 counties in South Carolina, 4 counties in Tennessee, 1 county in West Virginia, 19 counties in Missouri, and 2 counties in Oklahoma. These records indicate that for the past 40 years rosette has taken its toll of trees, and that it has spread over a considerable area.

IDENTITY OF THE DISEASE

In 1890–91, Smith[2] conducted experiments in middle Georgia in which he showed that peach rosette is an infectious disease. Of 125 seedling peach trees into which he inserted buds from a rosetted peach tree, 121 developed rosette.

In June, 1891, Smith[3] inoculated 37 Elberta peach trees with buds from a rosetted Kelsey plum. Two trees developed rosette and died in August, 1892. The other inoculated trees remained healthy. As a result of this experiment Smith said:

> The small per cent of cases to unions makes it necessary to repeat this experiment before it can be stated positively that the plum disease is identical with that of the peach and transmissible to it, as seems very probable from its appearance.

In June, 1891, Smith[2] inoculated 104 Marianna plum trees by inserting buds from rosetted peach trees. After 16 months not a single case of rosette had developed on the Marianna plums, so he concluded:

> There is, therefore, good reason to believe that the Marianna plum is not subject to this disease.

In June, 1891, 12 Marianna plum trees were inoculated by Smith[2] with buds from a rosetted Kelsey plum. On final examination in November, 1892, the Marianna plums showed no signs of rosette.

The presence of rosette in orchards at the Georgia Experiment Station, and in a number of commercial orchards in various sections of the State led to further study of this disease by the writer, beginning in 1919. As data presented by Smith[4] indicated that fungi and microscopic bacteria were not the cause of rosette, the writer did not attempt to repeat this phase of the work.

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[1] Accepted for publication Aug. 18, 1922.

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PEACH TO PEACH, PLUM, AND APRICOT

EXPERIMENT 1.—In the spring of 1919, rosette appeared on one side of a six-year-old seedling peach tree in an experimental orchard at the station. The other side of this tree appeared normal throughout the summer of 1919. During this time buds from healthy peach, plum, and apricot trees were inserted into the new growth of the normal appearing branches. The plum buds failed to unite with the peach stock. The peach and apricot buds united with the peach stock, but remained dormant throughout the summer. In the spring of 1920, the branches of the seedling peach which showed rosette in 1919, did not produce leaves, and examination showed that they were dead. The branches which appeared normal in 1919 produced rosetted growth in the spring of 1920. The peach and apricot buds inserted in 1919 produced typical rosetted shoots showing that the causal entity had passed from the diseased peach stock to the buds. The apricot buds produced shoots from two to three inches in length and the leaves had the mottled appearance of a mosaic disease. The mottled appearance was not so striking on the peach leaves. The shortened internodal growth, together with mottling of the leaves of some hosts and the absence of microscpic bacteria, put peach rosette in the class of virus or mosaic diseases. This experiment indicated that both the peach and the apricot are hosts for rosette.

PEACH TO PEACH

EXPERIMENT 2.—On June 10, 1919, buds from a healthy Elberta peach tree about 20 years of age were put in the new growth of two seedling peach trees about two years of age to serve as controls. On the same date buds from rosetted shoots of the seedling peach described in experiment 1 were put in the new growth of two peach seedlings the same age as the controls. On August 29, 1919, it was observed that the Elberta buds on the control trees had grown into healthy shoots. In one of the inoculated trees a diseased bud had developed a rosette of leaves, and below the point where this bud was inserted some of the buds of the stock had developed rosettes characteristic of this disease. In the other inoculated peach seedling the bud had remained dormant, but the buds of the stock immediately below the point of inoculation had developed typical rosettes. In May, 1920, it was observed that the two control trees were healthy and growing vigorously, while the inoculated trees had become completely rosetted (Pl. 1, A).

PEACH TO APRICOT TO PEACH

EXPERIMENT 3.—On August 15, 1919, two buds from a rosetted twig of the peach tree described in experiment 1 were put into the new growth of a healthy Royal apricot, which was in its second year of growth. The peach buds remained dormant until the spring of 1920 when they developed into rosetted shoots. The lateral buds on the apricot branch below the point of inoculation also developed weak shoots, but the leaves did not have the typical rosetted appearance. The internodal growth, although longer than that of the rosetted peach shoot (Pl. 2, A), was much less than that of healthy apricot trees growing near. Plate 2, B, shows the stunted growth of this Royal apricot 12 months after inoculation with peach rosette. While the growth is not typical of rosette as

it appears in the peach, the shortened internodal growth, and the mottling of the leaves indicate that the causal entity had been transferred from the peach to the apricot.

On September 2, 1920, two buds were taken from the rosetted Royal apricot and inserted in a peach seedling, growing in a pot in the greenhouse. These buds remained dormant until April, 1921, when they began to develop mottled leaves. The new growth of the peach seedling developed typical rosettes (Pl. 1, B). Both the potted rosetted peach seedling and the rosetted Royal apricot tree died during the summer of 1921.

Experiment 3 shows that the causal entity of rosette may be transferred from peach to apricot, and from apricot to peach, proving that apricot and peach rosette are identical as far as causal entity is concerned, but somewhat different in external manifestations of the disease.

STUNTING OF APRICOT GROWTH BY ROSETTE

EXPERIMENT 4.—In the spring of 1920 another case of natural infection of peach rosette (Pl. 3, A) developed in a 6-year-old seedling peach tree growing in the same orchard about 150 feet from the rosetted seedling described in experiment 1.

On June 19, 1920, buds from this second rosetted seedling peach were inserted into a branch of a one-year-old Moorpark apricot about a foot above the ground. By August 20, 1920, both of the peach buds had grown into rosetted shoots (Pl. 5, B), but the stock showed symptoms of rosette only in the growth immediately below the inserted peach buds, where the apricot leaves became mottled. An apricot limb adjoining the one which was inoculated, made a growth of 4 feet and 4 inches during the summer of 1920, and bore normal green leaves. There was no growth of lateral buds. On April 11, 1921, the lateral as well as the terminal buds on all the apricot branches had produced mottled, greenish yellow leaves, in marked contrast to the normal green leaves of the near-by healthy apricot. This indicated that the causal entity of rosette had spread throughout the apricot tree. During the summer of 1921 this Moorpark apricot tree grew very slowly, the maximum growth of any one branch being 5 inches. During the same time the near-by healthy apricot tree made a growth of 4 feet and 9 inches. Plate 3, B (taken in the fall of 1921) shows the inoculated apricot tree with a background, at the left, in contrast to the healthy apricot tree at the right. An indication of the stunting effect of rosette is shown by the inoculated tree in which the maximum vertical growth of 4 feet and 4 inches made during the summer of 1920 represents normal growth; while the maximum horizontal growth, 5 inches, represents development made during the summer of 1921 under the retarding influence of rosette.

PEACH TO CULTIVATED PLUM

EXPERIMENT 5.—On August 6, 1919, a bud from a healthy Blue Damson plum was placed in the trunk of a healthy peach seedling which came up in the spring of 1919. This plum bud made a few inches of growth during the summer of 1919, and in the summer of 1920, made a growth of more than 2 feet. Neither the plum branch nor the peach stock showed any symptoms of disease up to June 19, 1920, when two buds from the rosetted peach tree described in experiment 4, were inserted in

the Damson plum branch. The diseased peach buds started growth within two weeks, producing typical rosetted shoots, but with somewhat larger leaves and more internodal growth than did similar rosetted buds on the diseased peach stock from which the buds were taken. During the summer of 1920, the plum buds just below the place where the peach buds were inserted, developed small rosettes of mottled leaves (Pl. 4, A) indicating that the causal entity had passed from the diseased peach buds into the healthy Damson plum branch. None of the branches of the peach stock on which the plum was budded showed symptoms of rosette during the summer of 1920. The growth of the peach stock was so vigorous that a small copper wire, by which a label had been attached to the trunk in 1919, became embedded in the tissues of the stock, a few inches above the point where the Damson plum bud was inserted. In the spring of 1921, rosette developed in all growth of the Damson plum branch, and in all branches of the peach stock which grew from the trunk at points below where the copper wire was embedded in the tissues. None of these rosetted branches set fruit, though a few produced weak blossoms. All of the branches which grew from the trunk above where the wire was embedded produced normal leaves and blossoms which set numerous fruits. By July, 1921, the leaves on this tree began to wither, and by August 20 the tree was practically dead (Pl. 4, B). No symptoms of rosette appeared on the tree above the embedded wire, and the leaves and fruits shriveled and clung to these branches for some time after the tree was dead. The fact that no symptoms of rosette appeared above the embedded wire supplies additional data as to the tissues through which the causal entity progresses, and is being further investigated.

In this experiment rosette was transferred from the peach to the plum, and back to the peach, indicating that peach and plum rosette are identical.

Rosette has also been transferred from peach to Red June plum by means of infected buds.

PEACH TO WILD PLUM TO PEACH

EXPERIMENT 6.—On September 22, 1920, buds from a rosetted Mayflower peach, 7 years old, which developed as a natural infection in one of the station orchards in the spring of 1920, were put in two wild Chickasaw plum trees, growing in a fence row on the station. These buds remained dormant until the spring of 1921, when both the peach buds and the plum stock developed rosetted shoots. The inoculated plums (Pl. 4, C) grew more slowly than the surrounding healthy plum trees during the summer of 1921. The appearance of the rosetted wild plum is not so striking as that of a rosetted peach, because the plum is naturally of dwarfed growth. The wild plum, being of no economic importance, grows in waste places without coming under the close observation of man; therefore one or more rosetted wild plum trees might easily be an unobserved source of infestation to surrounding orchards.

On May 23, 1921, buds from the rosetted wild Chickasaw plum were inserted in the new growth of a 2-year-old seedling peach. During the summer one of these plum buds produced a rosetted shoot about an inch in length, but no symptoms of rosette appeared in the peach stock up to the time it was defoliated by frost. In the spring of 1922 this

peach seedling showed rosette in all new growth (Pl. 5, A). Peach seedlings into which healthy plum buds were inserted in 1921 showed no symptoms of rosette in the new growth of 1922.

This indicates that the casual entity of rosette may readily be transmitted from peach to wild plum, and from wild plum to peach.

Rosette has also been transmitted from the wild Chickasaw plum to the Red June plum by means of infected buds.

PLUM TO PEACH

EXPERIMENT 7.—A natural infection of a Maynard plum developed in an orchard on the station in the spring of 1920. By June 15, 1920, most of the lateral buds had grown into rosetted shoots from 1 to 3 inches long; and by August, 1920, this tree (Pl. 6, B) had made very little new growth as compared with a near-by healthy plum tree (Pl. 6, A). On June 18, 1920, buds from this rosetted plum tree were put into a healthy seedling peach tree in its second season's growth. Buds from a healthy plum were put into another peach seedling to serve as a control. On August 18, 1920, it was observed that some of the rosetted Maynard plum buds put into the peach seedling had produced shoots several inches long. Below the point where the diseased buds were inserted the peach buds had developed rosetted shoots (Pl. 6, C). The disease continued to spread in this peach seedling during the rest of the season of 1920, and when new growth started in the spring of 1921 this tree showed rosette in all parts. It died before midsummer. One of the control buds produced normal leaves (Pl. 7, A) and the peach stock on which it was growing was alive and healthy in the fall of 1921 when it was removed to make room for other experimental work.

This experiment indicates that rosette originating in the cultivated plum may be transmitted to the peach.

PEACH TO MARIANNA PLUM

EXPERIMENT 8.—On June 18, 1920, buds from the rosetted peach seedling described in experiment 4 were put into a healthy Marianna plum branch, near the base of the tree. One bud started growth within two weeks and produced a rosetted shoot (Pl. 7, B) with larger leaves and longer internodal growth than rosetted shoots on peach stocks. No signs of rosette appeared on the Marianna stock during the summer of 1920. After becoming dormant in the fall of 1920, this Marianna plum was transplanted to a large pot and placed in the greenhouse. In the spring of 1921 the peach shoot developed rosetted leaves, but continued to grow throughout the summer. The Marianna plum stock developed normal leaves on all of its branches, which grew rapidly throughout the summer. This Marianna plum (Pl. 7, C) had been under observation in the greenhouse during the winter, and up to May 1, 1922, it showed no symptoms of rosette. The rosetted peach shoot continued to grow slowly. When rosetted peach buds were put into a susceptible host, as peach, apricot, or ordinary cultivated plums, they died within 12 months. On the resistant Marianna plum stock the rosetted peach bud grew into a shoot which at the time this paper was written had lived for 22 months. This indicated that the resistant stock exerted a marked influence on the virulence of the causal entity of rosette in the peach scion.

PLUM TO MARIANNA PLUM

EXPERIMENT 9.—On June 18, 1920, buds from the rosetted Maynard plum described in experiment 7 were put into a Marianna plum branch, near the base of the tree. By September 18, 1920, one of the diseased buds had produced a rosetted shoot 8 inches in length with three branches from 3 to 5 inches in length (Pl. 8, A). This rosetted Maynard plum branch made considerably more growth on the Marianna stock than similar buds made on the susceptible Maynard stock, but the growth was decidedly rosetted and the leaves were mottled yellowish green. The Marianna stock grew vigorously throughout the summer of 1920 and showed no external symptoms of rosette.

Buds from a healthy Mayflower peach tree were put into the new growth of the Marianna stock on which the rosetted Maynard plum shoot was growing on September 18, 1920. These peach buds remained dormant until the spring of 1921, when two buds grew into healthy peach shoots. The Marianna plum branches and the Mayflower peach shoots made a vigorous growth during the summer of 1921, which showed no symptoms of rosette. The Maynard plum shoot made some growth during the summer of 1921, but at all times it had the characteristic symptoms of rosette. In the spring of 1922 the Marianna plum stock and the two Mayflower peach shoots (Pl. 8, B) developed normal leaves in contrast to the rosetted Maynard plum branch.

This experiment gave additional evidence that the Marianna plum is not susceptible to rosette. It also indicated that the causal entity of rosette does not pass from a host, such as the Maynard plum, through the tissues of the resistant Marianna plum stock to another susceptible host, as the Mayflower peach.

PEACH TO MAZZARD CHERRY

EXPERIMENT 10.—On June 19, 1920, buds from the rosetted peach tree described in experiment 4 were put into a healthy Mazzard cherry seedling about 1 year of age. On the same date buds from a healthy Elberta peach tree were put into a near-by Mazzard cherry tree of the same age to serve as a control. One of the rosetted buds united with the cherry stock and made a very feeble growth of rosetted leaves. The healthy buds united with the cherry stock but remained dormant. During the summer of 1920 the inoculated cherry stock grew slowly as compared with the control tree. The leaves of the inoculated tree became yellowish green and the new growth was small and in tufts similar to rosettes of peach leaves. The inoculated cherry stock developed leaves from both lateral and terminal buds in the spring of 1921, giving the new growth a decidedly rosetted appearance, especially at the tips of the branches. The control cherry stock produced vigorous new growth from the terminal buds. Very little growth was made by the diseased cherry tree during the summer of 1921, as shown by the smaller and more rolled leaves compared to those of the healthy control tree. The healthy Mazzard cherry control tree matured its buds and became dormant during the fall of 1921, while the diseased cherry tree attempted to make new growth from the terminal buds throughout the winter. By April 25, 1922, the inoculated tree (Pl. 9, A) was much stunted and had the appearance of being in an advanced stage of rosette, while the control

tree (Pl. 9, B) showed no symptoms of rosette and had made a vigorous growth.

This experiment indicates that the causal entity of peach rosette may be transmitted to Mazzard cherry and may produce symptoms similar to but not exactly the same as rosette of the peach. In the case of the Mazzard cherry there is evidently some resistance to the causal entity of rosette, for the infected cherry tree was alive June 1, 1922 (when this paper was written), 23 months after showing symptoms of rosette. Rosette has also been transmitted to two additional Mazzard cherry trees by means of infected peach buds.

WILD PLUM TO BITTER ALMOND

EXPERIMENT 11.—Through the courtesy of members of the California Agricultural Experiment Station, fresh seed of Bitter almond, and Texas Seedling almond were obtained and planted in the greenhouse on November 24, 1920. During April, 1921, some of the young almond trees were transplanted to the nursery. On May 23, 1921, buds from a rosetted wild Chickasaw plum (used in experiment 6) were put into two Bitter almond seedlings in the nursery. Three uninoculated trees of the same variety served as control. The plum buds united with the almond stocks and during the summer of 1921, one grew into a rosetted shoot about 6 inches long. Almond buds on the stock below the point where the rosette plum buds were inserted grew into small rosetted shoots which died during the winter. By May 24, 1922, the new growth of this inoculated tree was stunted (Pl. 10, A), and the leaves were yellowish green. The other inoculated tree had shown no marked symptoms of rosette at the time this paper was written. The adjoining uninoculated trees made a vigorous growth in the spring of 1922 and showed no symptoms of rosette.

This experiment indicates that the Bitter almond is susceptible to rosette.

APRICOT TO BITTER ALMOND AND TEXAS SEEDLING ALMOND

EXPERIMENT 12.—On April 18, 1921, buds from the rosetted Royal apricot of experiment 3 were put into one Bitter almond and one Texas Seedling almond growing in pots in the greenhouse. The buds united with the almond stocks, but made very little growth during the summer of 1921. Lateral buds on the two almond stocks, below the points of inoculation, developed small rosetted shoots indicating that the causal entity had been transferred from the rosetted apricot to both Bitter almond and Texas Seedling almond. Uninoculated almond trees of the two varieties growing in near-by pots remained healthy. In the spring of 1922 the inoculated trees became rosetted in all parts; the growth was stunted and the leaves were yellowish green. The uninoculated trees made a vigorous growth, however, and bore healthy green leaves.

This experiment proves that both the Bitter almond and the Texas Seedling almond are susceptible to rosette.

ALMOND TO PEACH

EXPERIMENT 13.—On February 2, 1922, buds were taken from the rosetted Bitter almond and Texas Seedling almond of experiment 12 and inserted in healthy peach seedlings growing in pots in the greenhouse.

One of the Bitter almond buds had produced a much branched shoot about 16 inches long by June 1, 1922, the leaves of which were more tufted in growth than those of healthy Bitter almonds. The peach shoots which grew from the stock below the point of inoculation had developed an upward rolling of their older leaves and most of the lateral buds had produced small rosettes of yellowish green leaves (Pl. 10, B).

The Texas Seedling almond buds remained dormant and up to June, 1922, the peach stock into which they were inserted had shown no symptoms of rosette. Peach seedlings budded to healthy almonds have remained healthy up to June 1, 1922.

This, together with the foregoing experiments, indicates that peach, apricot, plum, and almond are susceptible to rosette and that in all cases the causal entity is the same.

SOIL TRANSMISSION OF ROSETTE

EXPERIMENT 14.—Natural infection of rosette has been observed by the writer in Georgia on peach trees from 2 to 8 years of age. Where rosette develops in an orchard of young trees the question arises as to the advisability of setting a healthy tree in the place from which a diseased tree has been removed. It has been proved by Smith[5] that infection may be produced through inoculation of peach roots, so it seemed advisable therefore to test soil transmission.

Two 6-year-old peach trees, which had developed rosette in the spring of 1919, were dug up September 5 of the same year and removed from the orchard. Early in January, 1920, a healthy 1-year-old peach tree on peach stock was set in each hole. No attempt was made to remove fragments of roots left from the rosetted trees. The two transplanted trees made satisfactory growth during the summers of 1920 and 1921 showing no symptoms of rosette. This indicates that one may safely set a healthy tree in a place from which a rosetted tree has been removed.

THE TRANSMISSION OF ROSETTE BY MEANS OF SAP FROM DISEASED TREES

In rosette of the several species of Prunus there is a shortening of internodal growth and in some cases mottling of the leaves similar to mosaic diseases of vegetable and field crops. Numerous inoculations have been made in various parts of susceptible species of Prunus using methods known to be successful in transmitting mosaics of other plants. In no case has rosette been produced by transfers of sap from rosetted to healthy trees, but experiments along this line are being continued. The results obtained thus far confirm data presented by Smith[6] to the effect that under artificial conditions rosette is transmitted only when an organic union takes place between infected tissues, and tissues of a susceptible host. Thus in experiments conducted to date, rosette differs from other mosaics in the method of artificial transmission, indicating that the causal entity is somewhat different from that of other mosaics.

[5] Smith, Erwin F. ADDITIONAL NOTES ON PEACH ROSETTE. In Jour. Mycol., v. 7, p. 226-232. 1893.
[6] Smith, Erwin F. THE PEACH ROSETTE. *In* Jour. Mycol., v. 6, p. 143-148, pl. 8-13. 1891.

NATURAL TRANSMISSION OF ROSETTE

The development of rosette in trees, often at a considerable distance from any known source of infection, indicates that winged insects or birds may be associated with natural transmission of this disease.

Various insects are found associated with rosetted trees, the most abundant being the black peach aphis, *Anuraphis persicae niger* Smith. Numerous tests have been made by removing insects, including several species of beetles and leafhoppers from various parts of rosetted trees, and caging them on healthy peach and plum trees. In no case has rosette developed. At various times throughout the growing season for the past two years, numerous black peach aphids have been transferred from rosetted peach and plum trees to healthy peach, plum, cherry, apricot, and wild plum trees growing in cages. The colonies of aphids increased rapidly in size showing that they were under favorable conditions. In no case did rosette develop as a result of these aphid transfers. These tests indicate that the causal entity of rosette is not readily transferred by the types of insects which are known to carry mosaic virus of other plants.

The writer is of the opinion that further study will disclose the fact that an animal, other than man, is responsible for the dissemination of the causal entity of rosette. Therefore observations along this line are being continued.

SUMMARY

The data obtained from the foregoing experiments verify the findings of Smith to the effect that rosette is readily transmitted from peach to peach by infected buds.

Rosette has also been transmitted to two varieties of apricots, two varieties of cultivated plums, one wild plum, one cherry, and two varieties of almonds by means of infected buds.

On some hosts rosette produces a mottling of the leaves similar to mosaics.

The Marianna plum is immune to rosette.

Limited tests indicate that rosette is not soil-transmitted.

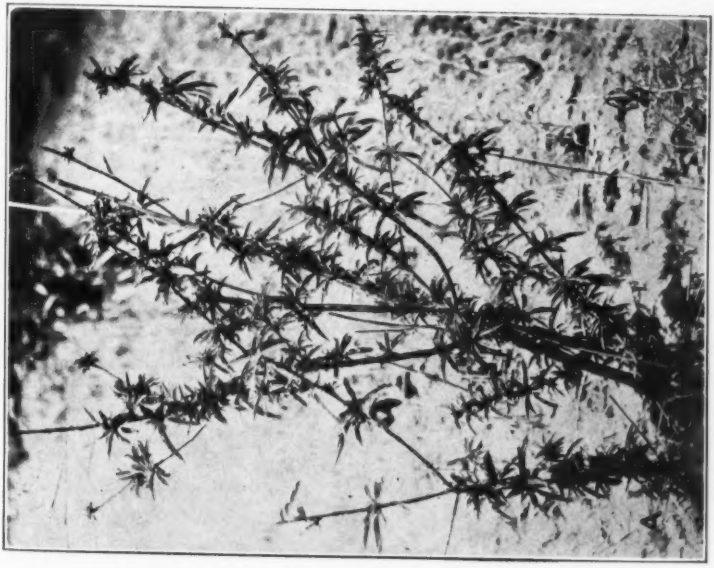
Numerous attempts to transmit rosette by means of sap from diseased trees has proved unsuccessful.

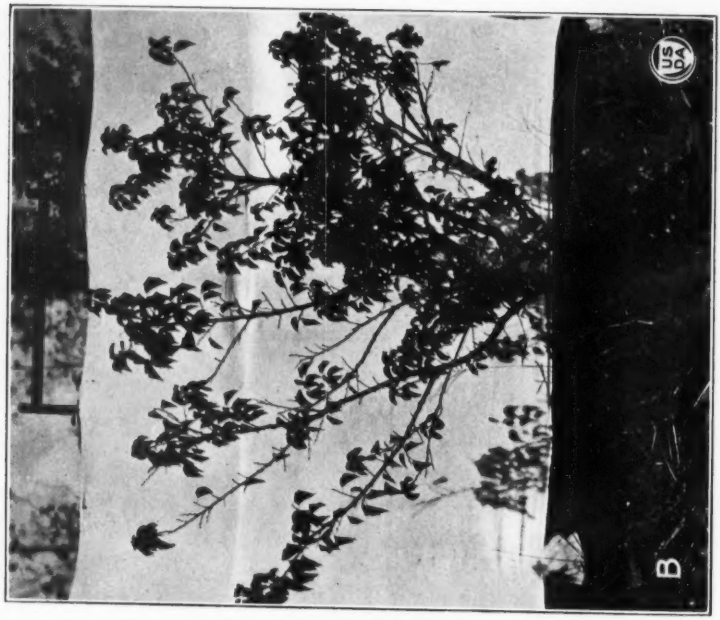
In a large number of transfers of various types of insects from rosetted trees to healthy susceptible hosts, not a single case of rosette was transmitted.

PLATE 1

A.—A 2-year-old peach tree into which buds from a rosetted peach were inserted June 10, 1919. Note the appearance in May, 1920, with all growth rosetted.

B.—The peach seedling into which buds from a rosetted Royal apricot were inserted September 2, 1920. Note the appearance in the summer of 1921, with all growth rosetted.




B

A

PLATE 2

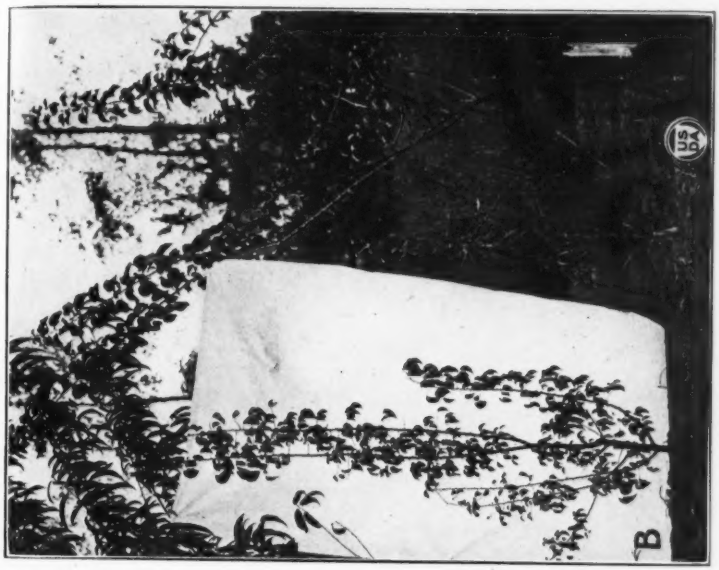
A.—A Royal apricot tree into which buds from a rosetted peach were inserted August 15, 1919. Note the rosetted peach shoot, and the stunted growth of the apricot shoots, made in 1920.

B.—The Royal apricot tree inoculated with peach rosette August 15, 1919. Note the stunted appearance of the whole tree on September 2, 1920.

PLATE 3

A.—A 6-year-old peach tree which developed a natural infection of rosette in the spring of 1920. Note the rosetted growth on the left in comparison with the apparently normal growth of two limbs on the right. During the summer of 1920 this tree developed symptoms of rosette in all parts.

B.—To the left, with a background, the Moorpark apricot shown in Plate 5, B, which was inoculated with peach rosette, June 19, 1920. Note the stunted growth of this tree in the fall of 1921, in comparison with the healthy apricot tree, to the right, without a background.


B

A

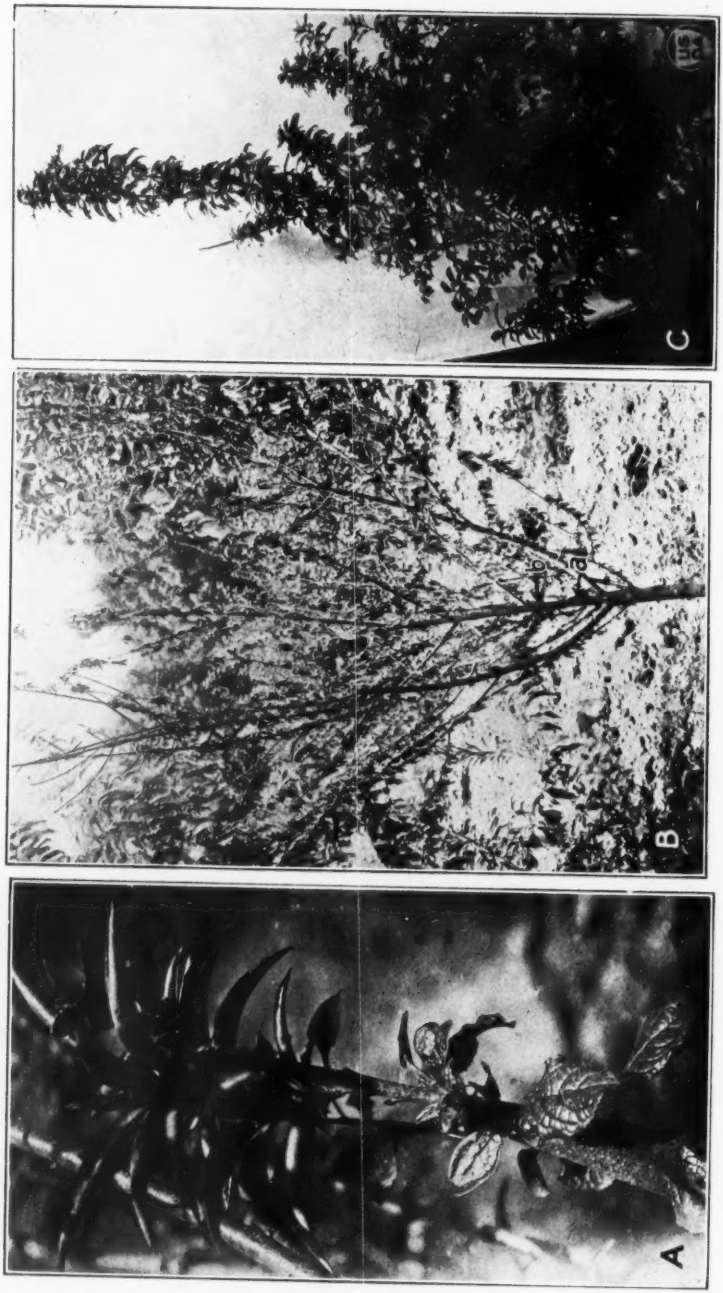

C
B
A

PLATE 4

A.—The Blue Damson plum branch into which rosetted peach buds were inserted June 19, 1920. Note the rosetted and mottled plum leaves just below the rosetted peach shoots.

B.—The peach seedling which was infected with rosette through a Blue Damson plum branch inoculated with buds from a rosetted peach. The arrow at *a*, shows the point on the peach stock from which the plum branch grew from a healthy bud inserted in 1919. The arrow at *b*, shows the location of the embedded copper wire above which the causal entity of rosette did not go. Note the condition August 20, 1920, with the leaves and fruits shriveled and dying.

C.—One of the wild Chickasaw plum trees into which rosetted Mayflower peach buds were inserted September 22, 1920. Note the stunted growth and the rosetted condition of this plum tree on September 2, 1921.

PLATE 5

A.—A seedling peach into which buds from a rosetted wild Chickasaw plum were inserted May 23, 1921. Note the completely rosetted condition of the peach seedling in May, 1922.

B.—A Moorpark apricot into which buds from a rosetted peach were inserted June 19, 1920. Note the rosetted shoots produced by the peach buds at *b*, and the apparently healthy growth of the apricot.

A
B
b
b
USDA

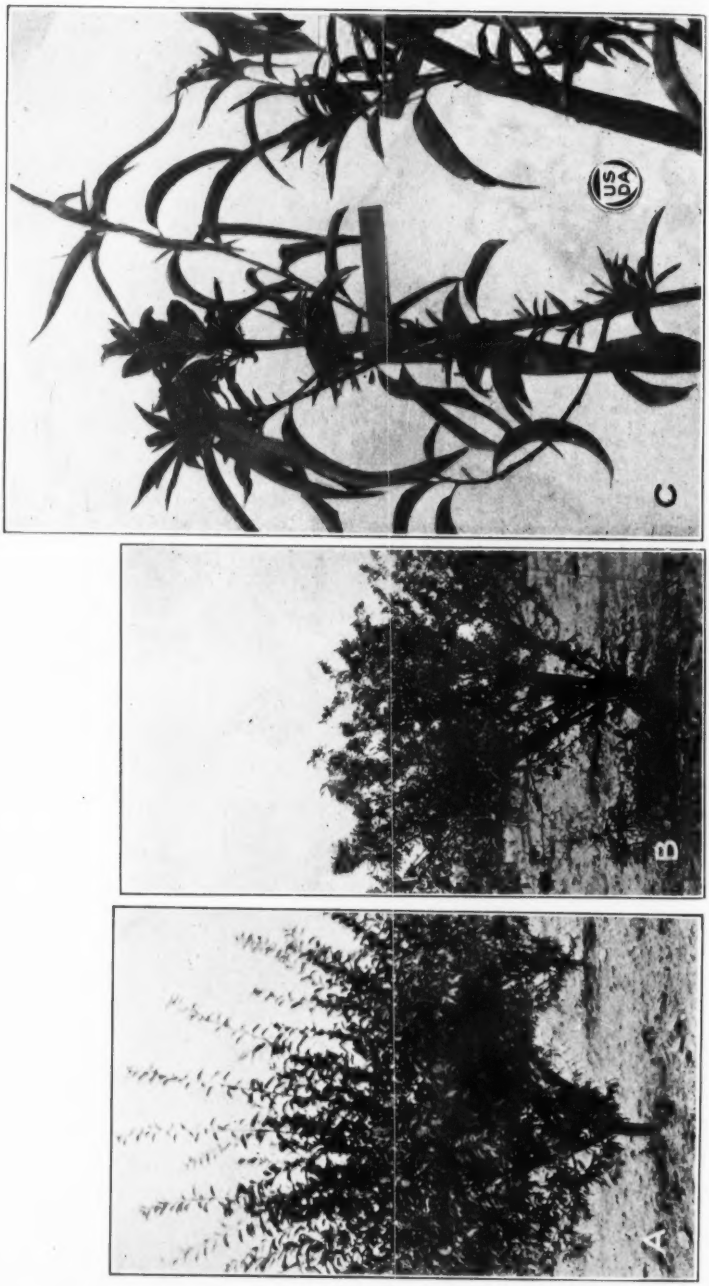

C
B
A

PLATE 6

A.—A healthy plum tree showing the vigorous growth made by August, 1920.

B.—The Maynard plum which developed rosette in the spring of 1920. Note the stunted growth and rosetted leaves which developed by August, 1920.

C.—The peach seedling into which rosetted Maynard plum buds were inserted June 18, 1920. Note the rosetted plum shoot which developed from one of the buds, and the rosetted peach shoots which developed as a result of inoculation with infected plum buds.

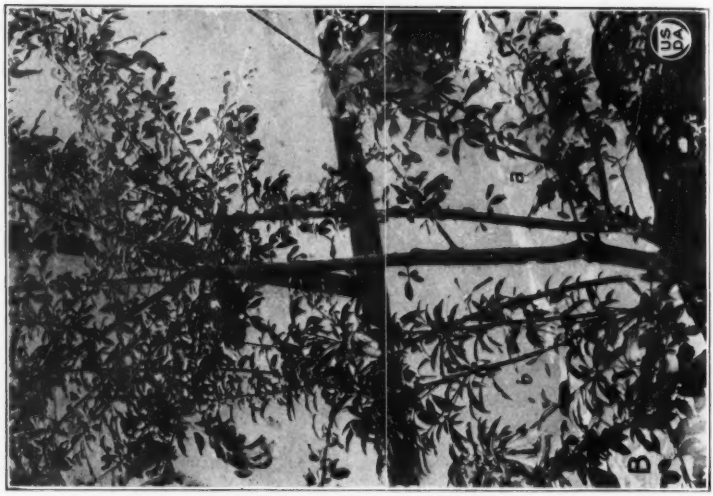
PLATE 7.

A.—The peach tree into which healthy plum buds were inserted June 18, 1920, to serve as a control. Note the healthy growth of the peach stock, and of one plum bud (indicated by the arrow).

B.—The Marianna plum tree into which buds from a rosetted peach seedling were inserted June 18, 1920. Note the rosetted shoot produced by one of the peach buds.

C.—The Marianna plum tree, shown in B, after another season's growth. Note the vigorous, healthy growth of the Marianna stock in contrast to the stunted growth of the peach shoot at *a*, with small rosettes at the tips of the branches.

C
a
USDA
B
A


B

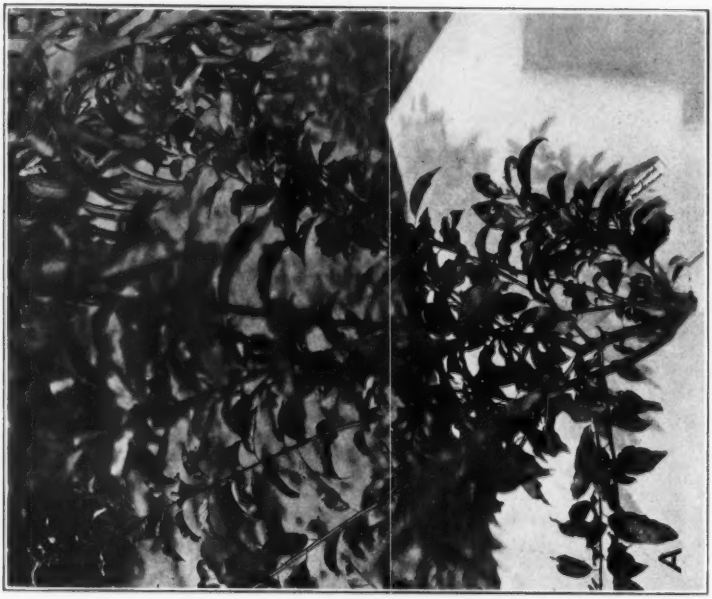

A

PLATE 8

A.—The Marianna plum tree into which buds from the rosetted Maynard plum were inserted June 18, 1920. Note the vigorous growth of the Marianna branches in contrast to the rosetted shoot, at the right, produced by one Maynard bud after three months' growth.

B.—The Marianna plum stock (same as in A but from the opposite side of the tree), showing the healthy growth of the two Mayflower peach shoots at *a*, in contrast to the stunted, rosetted Maynard plum shoot at *b*.

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PLATE 9

A.—The Mazzard cherry stock into which buds from a rosetted peach seedling were inserted June 19, 1920. Note the stunted growth and rosettes of leaves.

B.—The Mazzard cherry stock of the same age as A, into which healthy Elberta buds were inserted as a control.

A
B
U.S.D.A.

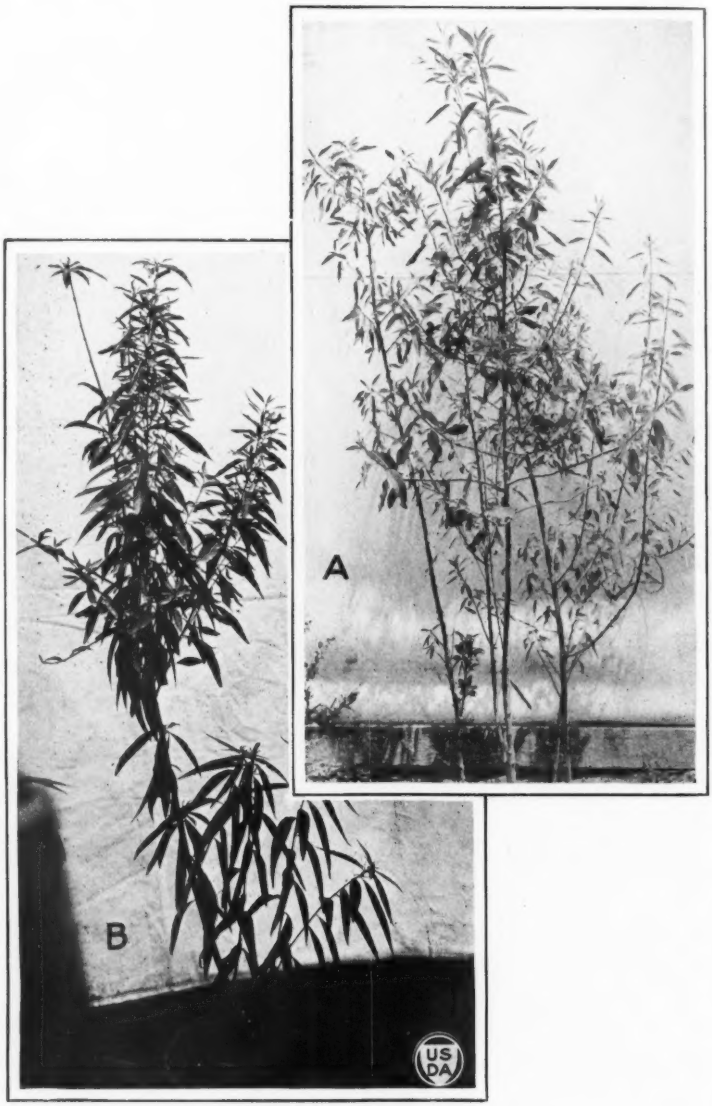
A
B
USDA

PLATE 10

A.—Bitter almond seedlings growing in the nursery. Note the stunted growth of the tree to the left with a rosetted plum shoot near the base, on May 24, 1922. This tree was inoculated May 23, 1921, with infected wild Chickasaw plum buds. The uninoculated seedlings to the right are healthy, and growing vigorously.

B.—A seedling peach tree into which a bud from a rosetted Bitter almond was inserted February 2, 1922. Note the much branched and tufted growth of the almond shoot which developed from the diseased bud. All the peach shoots which have developed below the point of inoculation have upward rolled leaves, and the lateral and terminal buds have produced small rosettes of yellowish green leaves.

TOXICITY AND ANTAGONISM OF VARIOUS ALKALI SALTS IN THE SOIL[1]

By F. S. HARRIS, *formerly Director*, M. D. THOMAS, *Associate in Agronomy*, and D. W. PITTMAN, *Instructor in Agronomy, Utah Agricultural Experiment Station*[2]

In the studies of soil alkali which have been carried out at the Utah Station (*4*, *5*)[3] during the past 10 years, a large number of the factors which influence the toxicity of most of the commonly occurring alkali salts have been correlated. It has been frequently noticed, however, that the toxicity of a mixture of salts in a soil seems to be the sum of the separate toxicities of the constituents of the mixture, and since these observations are at variance with the marked antagonistic action of the same salts in solution cultures a more detailed study of this question has been undertaken.

The experiments described in this paper were planned to show the influence on plant growth of adding other salts, as well as acids and manure, to a soil already impregnated with sodium carbonate. The possibility of finding a marked antagonism between some of these added substances and "black alkali" was an incentive to make the scope of the investigation as broad as possible. Since the results from this point of view, however, are largely negative the data are presented as a contribution to the literature on the toxicity of mixtures of alkali salts. Some preliminary work has also been done on soils impregnated with sodium chlorid and sodium nitrate instead of sodium carbonate.

REVIEW OF THE LITERATURE

Experiments conducted by Kearney and Cameron (*6*) were among the first to show the ameliorating effect of adding a second alkali salt to a solution which was already toxic to plants. In their work the plants were germinated while not in contact with the alkali salts, and the roots were then held in the solution for 24 hours. The toxicity of the solution, was determined by slight injury to the root tips. The toxic concentrations were not proportional to the concentrations which absolutely prevented growth of the plants, and the toxicity of the salts exchanged places somewhat when the absolute rather than the minimum check in growth was considered. Very small quantities of salts caused slight injury to the roots. The addition of a second cation to a toxic solution was found to reduce the toxicity of the solution more than the addition of a second anion. Sodium in most combinations greatly weakened the toxic action of magnesium. Calcium, especially in the form of the sulphate, markedly counteracted the injurious effect of either sodium or magnesium ions. Calcium sulphate was much more beneficial to the sulphates of sodium and magnesium than to their chlorids. A very

[1] Accepted for publication July 11, 1922.
[2] The authors wish to express their appreciation to Mr. N. I. Butt for his help in calculating and tabulating the data presented in this paper.
[3] Reference is made by number (italic) to "Literature cited," p. 337-338

effective neutralization of sodium carbonate injury was found when calcium chlorid was added to the solution.

The work of Miyake(*11*) with rice in cultures of magnesium sulphate, magnesium chlorid, calcium chlorid, sodium sulphate, and sodium chlorid showed that while *N/10* solutions of the individual salts were toxic, when two of these solutions were mixed in certain proportions the toxic effect was more or less neutralized. The greatest neutralization of toxicity was observed when calcium chlorid was added to magnesium sulphate or chlorid. Some antagonism was also noticed between chlorids and sulphates of sodium and magnesium, as well as between potassium chlorid and magnesium or calcium chlorid. In a study of the antagonism between sodium and potassium, the salts—sodium nitrate, potassium chlorid, potassium nitrate, sodium chlorid, potassium sulphate, and sodium sulphate—were used. The antagonistic action of the cations on each other was much greater than that of the anions. The greatest antagonism between sodium and potassium usually occurred when one part of one *N/10* solution was mixed with four parts of another.

Osterhout(*12*) found that antagonism between sodium chlorid and potassium chlorid was greater when one of the salts predominated in the solution than when both were present in nearly equal quantities. Small quantities of ammonium chlorid, magnesium chlorid, or calcium chlorid reduced the toxicity of either potassium chlorid or sodium chlorid, but with calcium chlorid even larger quantities were beneficial.

Kearney and Harter(*7*) tested the tolerance of eight different kinds of plants to solutions of sodium and magnesium salts and found that calcium sulphate greatly diminished the toxicity of the salts, especially magnesium. Calcium sulphate changed the order of toxicity of the solutions.

Hansteen(*3*), working with wheat seedlings, has found that calcium compounds exert a beneficial effect on the toxicity of solutions of alkali salts.

A comparison of soils and solutions by Harris(*4*) showed the antagonism between the common alkali salts to be more pronounced in solution cultures of wheat seedlings than in loam soils. In fact the only consistent case of antagonism observed in soil seems to have been with the nitrates of potassium, sodium, and magnesium at 4,000 parts per million (*4, p. 46*).

Some of the most positive antagonistic results in soils have been secured by measuring the activity of soil bacteria. By this means it has been shown by different experimenters that there is antagonism between anions as well as between cations of the salts common in alkali soils. This work has been so well summarized in a paper by Greaves(*2*) that the reader is referred to this publication for a review of this phase of the subject.

Experimenting with barley growing on a clay-adobe soil, Lipman and Gericke(*8*) found antagonism between sodium chlorid and sodium sulphate and between sodium chlorid and sodium carbonate in the second crop, though there was no antagonism shown between these salts in the crop grown soon after the salts had been added. A slight antagonism was noticed between sodium carbonate and sodium sulphate in the first crop. A marked antagonism between sodium sulphate and calcium sulphate was apparent in both the first and second crops.

To discover the possibilities of applying the principles of antagonism to the correction of alkali as found in field soils, Lipman and Sharp(*10*) secured natural alkali soil containing 6,400 parts per million of water-soluble salts of which 4,590 parts per million were sodium chlorid, 980 parts per million sodium sulphate, and 830 parts per million sodium carbonate. Adding 119 parts per million of sulphuric acid to this soil was found to be especially beneficial to the growth of barley; and up to 451 parts per million, the highest quantity tried, this acid was helpful. Calcium sulphate at the rate of 670 parts per million, ferrous sulphate at 324 parts per million, and manure at 3,240 parts per million all materially improved the crop-producing power of the soil. Copper and sodium sulphates at the rate of 65 and 130 parts per million, respectively, were harmful to the crop.

Lipman and Gericke(*9*), growing barley on a clay-adobe soil, found that copper and zinc reduced the toxicity of sodium chlorid, sodium sulphate, and sodium carbonate. Marked antagonism was also noticed between these salts, especially between copper sulphate and sodium chlorid when applied to a sand soil.

Caldwell(*1*) found no antagonism between sodium chlorid and any one of the chlorids of calcium, magnesium, potassium, copper, or ammonium, with the possible exception of ammonium and magnesium in certain proportions, when he grew corn in quartz sand. Potassium and sodium were always more toxic together than when only one was present at a given strength. Adding either calcium chlorid or copper chlorid to sodium chlorid appeared to ameliorate the injurious effect of the latter by diluting the solution rather than by counteracting the harmful effects of the sodium salt.

EXPERIMENTAL WORK

METHOD

The experiments here reported were conducted in glass tumblers containing the equivalent of 200 gm. of dry soil and the optimum amount of moisture. Wheat was the crop grown. The requisite quantity of sodium carbonate in 10 per cent solution was added to five 7-kilo portions of air-dry soil, and each portion was mixed separately by forcing it through a fine sieve twice. They were then placed together in a large can and shaken thoroughly at intervals for several days before being used. This method of mixing gave a very satisfactory distribution of the carbonate, as was shown by analyses of a large number of samples taken from different parts of the can. To the soil for each tumbler the other materials were then added, in solution whenever possible, together with the necessary water, and the soil was thoroughly mixed on a piece of oilcloth. Ten kernels of wheat were planted in each vessel. The surface of the soil was covered with a thin layer of a mixture of 50 per cent paraffin and 50 per cent petrolatum to prevent surface evaporation. A short glass tube extending to the center of the soil mass from the surface permitted the addition of water as it was needed to keep the weight of the tumbler constant. The mulch was very effective, and it was therefore possible to avoid altering the uniformity of the distribution of the soluble material through irrigation—at least until the plants were fairly large—as the weight of the tumbler did not change appreciably

at first. The plants which came up were counted daily and every four or five days their height was recorded also. At the end of 21 days they were measured for the last time, cut off at the surface of the ground, and dried to constant weight at 100° F. Data were thus secured on the days to germinate, the average height, and the dry weight of the plants. Most of the discussion in this paper, however, will be based on the dry weight which is perhaps the most reliable index of growth.

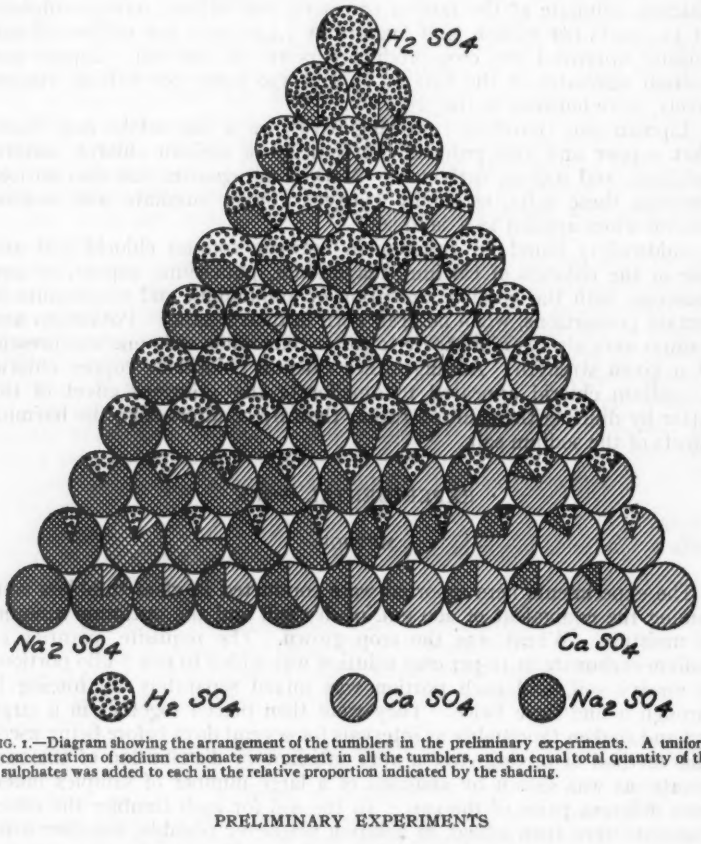

FIG. 1.—Diagram showing the arrangement of the tumblers in the preliminary experiments. A uniform concentration of sodium carbonate was present in all the tumblers, and an equal total quantity of the sulphates was added to each in the relative proportion indicated by the shading.

PRELIMINARY EXPERIMENTS

During the summer of 1919 some preliminary work was done on this problem. The technic of the experiment was essentially the same as that described above, except that no mulch was used and the preliminary mixing of the carbonate with the soil was not quite so thoroughly done. The tumblers were arranged in a triangular formation consisting of 66 glasses. All contained a single concentration of sodium carbonate together with sodium sulphate, calcium sulphate, and sulphuric acid in the different concentrations represented in figure 1. The total concentration in parts per million of the added sulphates was always the

same as that of the carbonate, but there were 10 different quantities of each individual sulphate in the triangular grouping. In addition, there were 12 extra tumblers containing the four added substances alone, at their maximum concentrations. A "trial" consisted of 10 of these treatments in which the quantity of carbonate and sulphates added ranged from 1,000 to 10,000 parts per million. Three trials were first carried out with these materials in sand, Greenville loam, and West Logan clay loam. Then two more trials were made with Greenville loam and the same sulphates but with the substitution of sodium chlorid in one case and sodium nitrate in the other in place of sodium carbonate. In the last two trials the concentrations of the salts and also the sulphates ranged from 500 to 5,000 parts per million.

Mechanical and partial chemical analyses of the soils used in these experiments have already been published by one of the writers (*13*). All the soils contained a large quantity of calcium carbonate. The sand was coarse and low in organic matter. The two heavier soils were nearly identical in texture, but the Greenville soil contained a little more organic matter.

A critical study of the results of this preliminary test has shown that the experimental error in the individual tumblers may frequently be large enough to destroy the regularity of the variations due to the gradual interchanging of the three sulphates so that a presentation of the data in full detail would be confusing. The triangular formation has therefore been divided into seven regions, namely, the center, the three corners, and the middles of the three sides. The value for each region has been found by averaging three to six tumblers. It is fully realized that no two tumblers had exactly the same "alkali" treatment, and, accordingly, this mode of presentation may not seem strictly justifiable, but since it has been observed that the average for three glasses in a given area is usually rather close to the average of six or more tumblers at that place, it is felt that this objection is of minor importance. The results are reported on the basis of the average dry weight per plant. The use of this basis neglects the fact that the percentage of germination in the higher concentrations of alkali is very much reduced and therefore the curves do not fall as rapidly as they would on the basis of total dry-weight production. The results seem quite comparable, however, and, as the relations in the higher concentrations are thus more clearly brought out, it has seemed best to adopt this basis. The data are given graphically in figures 2 to 6, each of which consists of four charts. Chart A shows the results of the sulphuric acid treatment with and without sodium carbonate, while B and C give the corresponding data for calcium sulphate and sodium sulphate, respectively. Finally, the two and three component mixtures added to the carbonate soil are shown in Chart D. The data for the untreated carbonate soil is reproduced in all the charts as a heavy unbroken line.

The results of the experiment with sand are given in figure 2. The beneficial action of calcium sulphate (fig. 2, B) and sulphuric acid (fig. 2, A) on the carbonate soil is forcibly shown. The acid is somewhat more efficacious than the calcium sulphate, as would be expected from the fact that the gypsum is too insoluble to be added in solution and hence would react more slowly. Since the sand contained a large quantity of calcium carbonate, some calcium sulphate was doubtless formed on the addition of the acid, and it therefore seems likely that both correc-

tives functioned in the same way. Sodium sulphate showed a very slight ameliorating action at 1,000 parts per million, at which concentration it appears to be decidedly stimulating to plant growth when added alone, but at higher concentrations it increased the toxicity of the carbonate (fig. 5, C). The mixtures of equal parts of two or three sulphates were all somewhat beneficial, but the presence of sodium sulphate noticeably reduced the corrective power of the mixture in proportion to its concentration (fig. 5, D). All the observations that have been made with the sandy soil can be readily explained by the simple ionic reactions between the added substances, and, accordingly, this case is not essentially different from a solution culture.

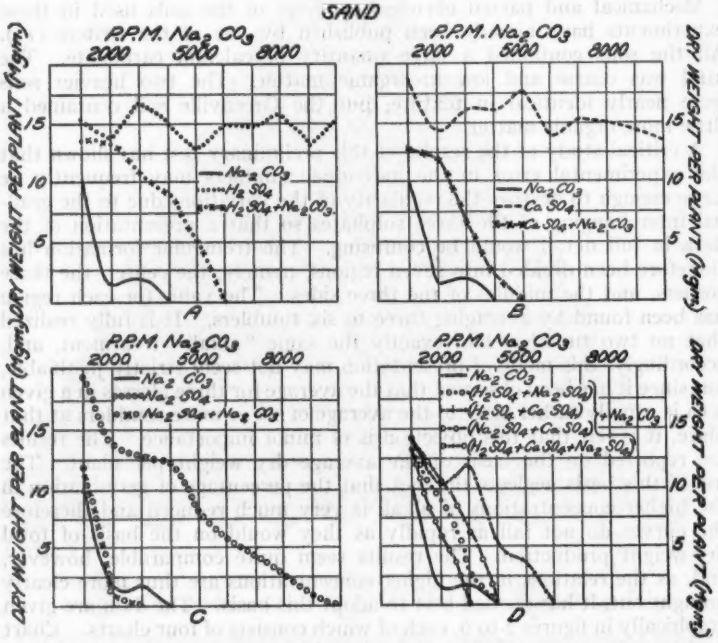

FIG. 2.—Diagram showing the effect on the growth of wheat plants of adding sulphates to sand impregnated with an equal total quantity of sodium carbonate.

In figures 3 and 4 are shown the results obtained when the previously mentioned materials were added to Greenville loam and West Logan clay loam, respectively. Both soils behave similarly and may be conveniently discussed together. As in the case of sand, neither the sulphuric acid nor the calcium sulphate is toxic at any of the concentrations used. The sodium sulphate begins to reduce the growth of the plants appreciably at about 5,000 parts per million, and the mixtures of this salt with sodium carbonate show decided additive toxicity except possibly at the lowest concentrations. For example, the dry weight per plant in Greenville soil is reduced to one-half normal (6.7 mgm.)

by sodium carbonate alone at 7,500 parts per million, by sodium sulphate at 9,500 parts per million, and by a mixture of the two containing 5,000 parts per million each (fig. 3, C). Considering the fact that there is some chemical removal of these salts from the soil solution by the soil material (*13, p. 431*) so that their concentration is really lower than the amount added indicates, these toxicity relations appear to be very nearly additive.

All the other mixtures follow the untreated sodium carbonate curve much more closely, indicating that they have very little, if any, influence on the toxicity of this salt, though the presence of sodium sulphate in

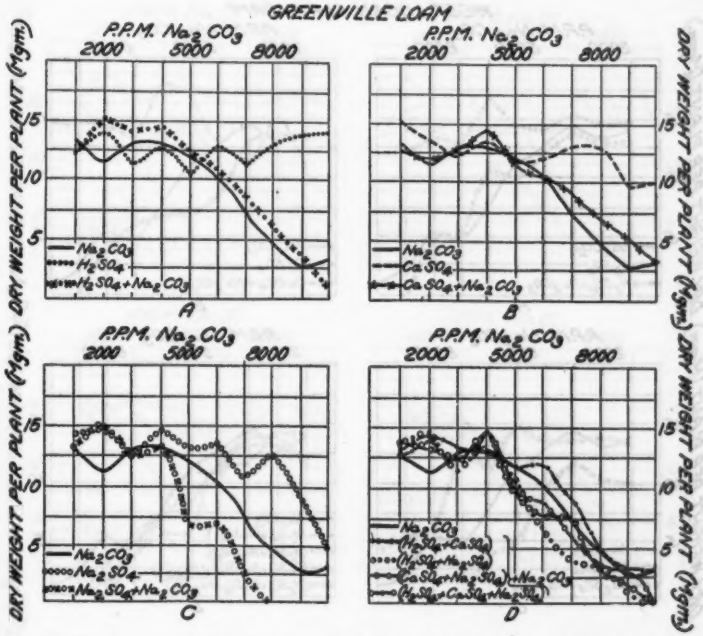

FIG. 3.—Diagram showing the effect on the growth of wheat plants of adding sulphates to Greenville loam already impregnated with an equal total quantity of sodium carbonate.

them is nearly always made manifest by the somewhat lower position of the curve. It is interesting to note that the calcium sulphate when mixed with sodium carbonate does not react in the same way in both soils. In the Greenville soil it shows no harmful effects at all in conjunction with the carbonate, whereas in the West Logan soil it seems to lower the yield slightly. There is certainly no striking evidence of antagonism between any of these substances in either of the two heavier soils.

Figure 5 represents the data for the dry weight per plant obtained by treating the Greenville soil, already impregnated with sodium chlorid, with sulphuric acid, calcium sulphate, and sodium sulphate. The con-

centrations of all these substances ranged from 500 to 5,000 parts per million. The general relations are nearly the same as with the carbonate shown in figures 3 and 4. The simple mixture of sodium chlorid and sodium sulphate shows additive toxicity, while the other mixtures add a much smaller amount or nothing at all to the harmful effects of the chlorid. The three and four component mixtures follow the simpler chlorid curve closely (fig. 5, D). No antagonism for sodium chlorid is evident.

The arrangement of the experiment represented in figure 6 differs from that of figure 5 only in having sodium nitrate substituted for

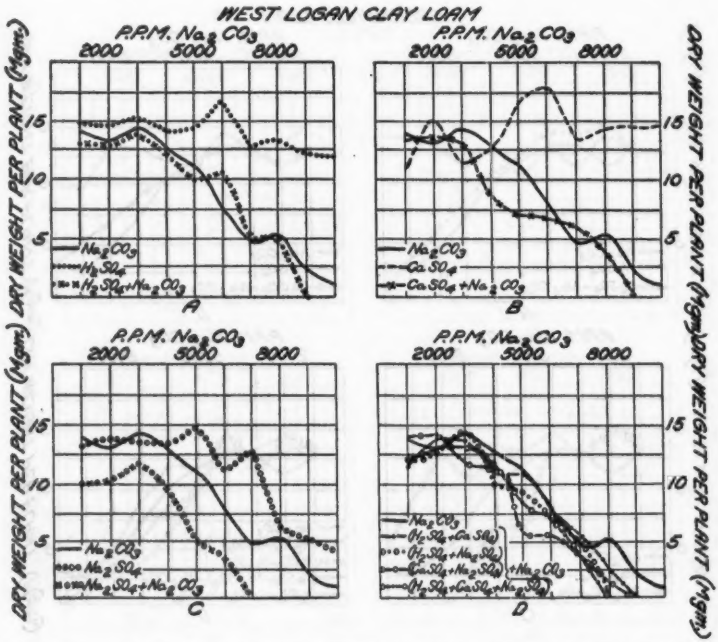

FIG. 4.—Diagram showing the effect on the growth of wheat plants of adding sulphates to West Logan clay loam already impregnated with an equal total quantity of sodium carbonate.

sodium chlorid. The relative shapes and positions of all the curves are so exactly analogous to those in figure 5 that the remarks and conclusions given above apply in this case also.

LATER WORK

With the experience gained in the experiments already described, the investigation of this problem was continued in the summer of 1921. It was decided to concentrate effort on one soil, containing one salt as a base, and to vary the subsequent treatment as much as possible. Accordingly, six different trials were carried out with Greenville loam

as the soil and sodium carbonate as the common salt in all the mixtures. In each trial three different substances were added, so that the effects of 15 materials in conjunction with sodium carbonate have been studied.

The triangular arrangement of the experiment was adhered to, but the number of individual treatments was reduced from 66 to 15, as shown in figure 7. In addition there were included in each triangular arrangement five control treatments in which each of the four individual materials and also distilled water were added to the soil alone. The whole experiment was done in duplicate. The same sodium carbonate treatment was present in five of these triangular groupings in which the concentrations

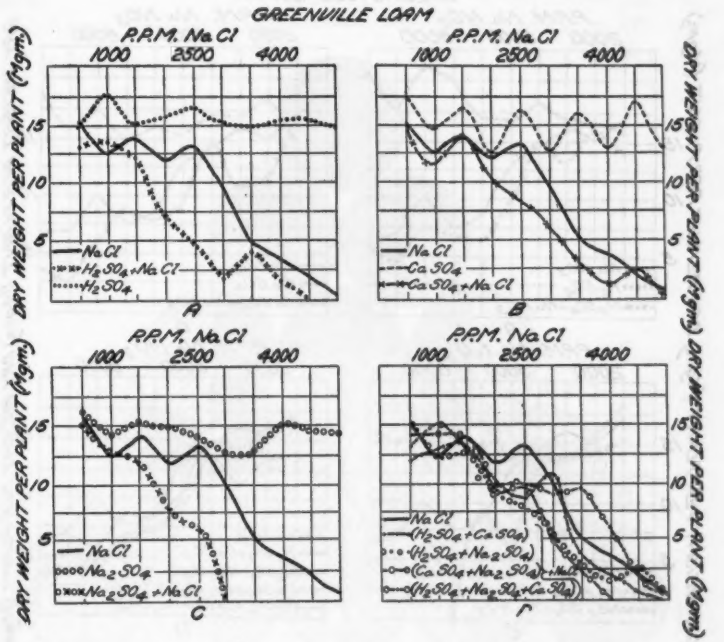

FIG. 5—Diagram showing the effect on the growth of wheat plants of adding sulphates to Greenville loam already impregnated with an equal total quantity of sodium chlorid.

of the other added substances, when they were toxic in themselves, were made to range as nearly as possible from stimulating amounts to decidedly toxic quantities. Four concentrations of sodium carbonate ranging from 1,000 to 7,500 parts per million made up a trial, which thus consisted of 20 units containing 40 tumblers each.

In figures 8 to 13 the results of six trials are presented graphically on the basis of the dry weight per plant. The straight dotted line across all the charts gives the yield secured from the untreated soil, as derived from 40 tumblers. The heavy unbroken line shows the yield from the soil treated with sodium carbonate alone and is the average of 10 tumblers.

It will be noticed that every concentration of sodium carbonate is represented in the figure by three charts containing six curves in each. On

the left is shown the results of mixing the three added substances at their five maximum concentrations with the untreated soil and also with the carbonate soil (fig. 7, tumblers 1, 11, and 15). The center chart represents tumblers 2, 3, 7, 10, 12, and 14 (fig. 7). These contain sodium carbonate and a binary mixture with one of the added substances distinctly predominating. On the right are given the results for tumblers 4, 5, 6, 8, 9, and 13 (fig. 7). These contain the carbonate and both binary and ternary mixtures in which the concentrations are more nearly equal. It should be emphasized that the fractions occurring in the legends to

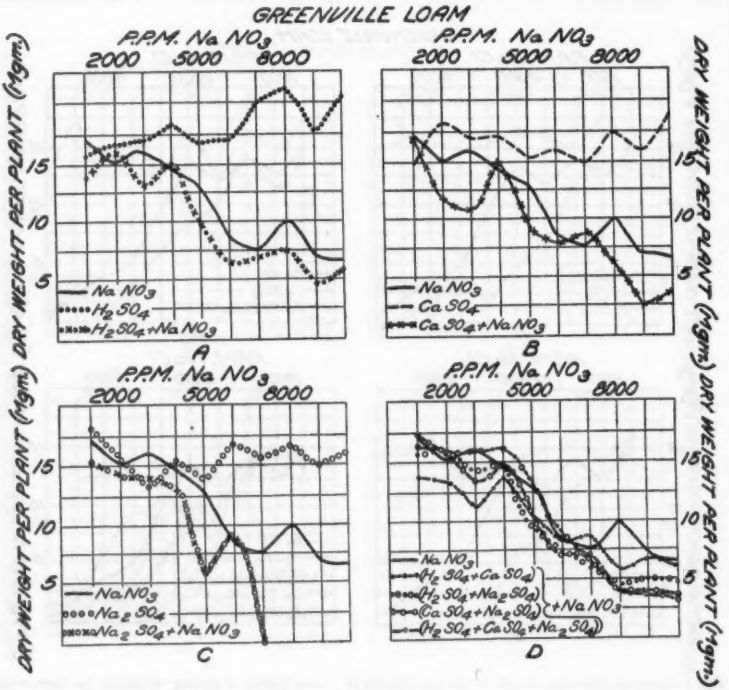

FIG. 6.—Diagram showing the effect on the growth of wheat plants of adding sulphates to Greenville loam already impregnated with an equal total quantity of sodium nitrate.

describe mixtures refer to a proportionate part of the maximum concentration of the unmixed substance and not to the total amount of the mixture. For example, in figure 8, in the second triangle, the maximum quantities of sulphuric acid, potassium sulphate, and sodium sulphate are 4,000, 3,000, and 3,000 parts per million, respectively. Then the mixture one-fourth sulphuric acid, one-half potassium sulphate, and one-forth sodium sulphate in this grouping contains 1,000 parts per million sulphuric acid, 1,500 parts per million potassium sulphate, and 750 parts per million sulphate, or a total of 3,250 parts per million, and it is this total which is plotted in the curves for this mixture.

SULPHURIC ACID, POTASSIUM SULPHATE, AND SODIUM SULPHATE

The concentrations used in this trial (fig. 8) are as follows:

	Parts per million at concentration—				
	1	2	3	4	5
H_2SO_4	2,000	4,000	6,000	8,000	10,000
K_2SO_4	1,000	3,000	5,000	8,000	10,000
Na_2SO_4	1,000	3,000	5,000	8,000	10,000
Na_2CO_3	1,000	2,000	4,000	6,000	.....

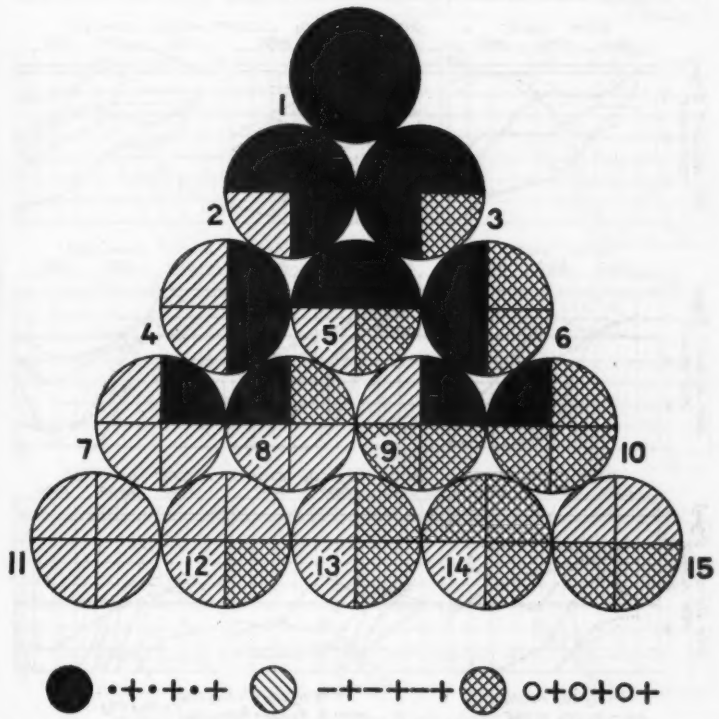

FIG. 7.—Diagram showing the arrangement of the tumblers in the final experiments. A uniform concentration of sodium carbonate was present in all the tumblers and the treatment was added as indicated by the shading. The maxium concentrations used are given in the text.

When added to the untreated soil, the potassium sulphate is only slightly toxic at 8,000 and 10,000 parts per million; the sodium sulphate is very detrimental to plant growth at 8,000 parts per million, and the acid is not harmful at all at 10,000 parts per million. This is indicated by the left section of figure 8, which also shows that the presence of sodium carbonate in the soil lowers the positions of these curves in proportion to its amount. The divergence of these pairs of curves due to

sodium carbonate also increases somewhat as the concentration of the sulphates increase. It is curious that this divergence should be appreciable when the quantity of carbonate present is not sufficient to show

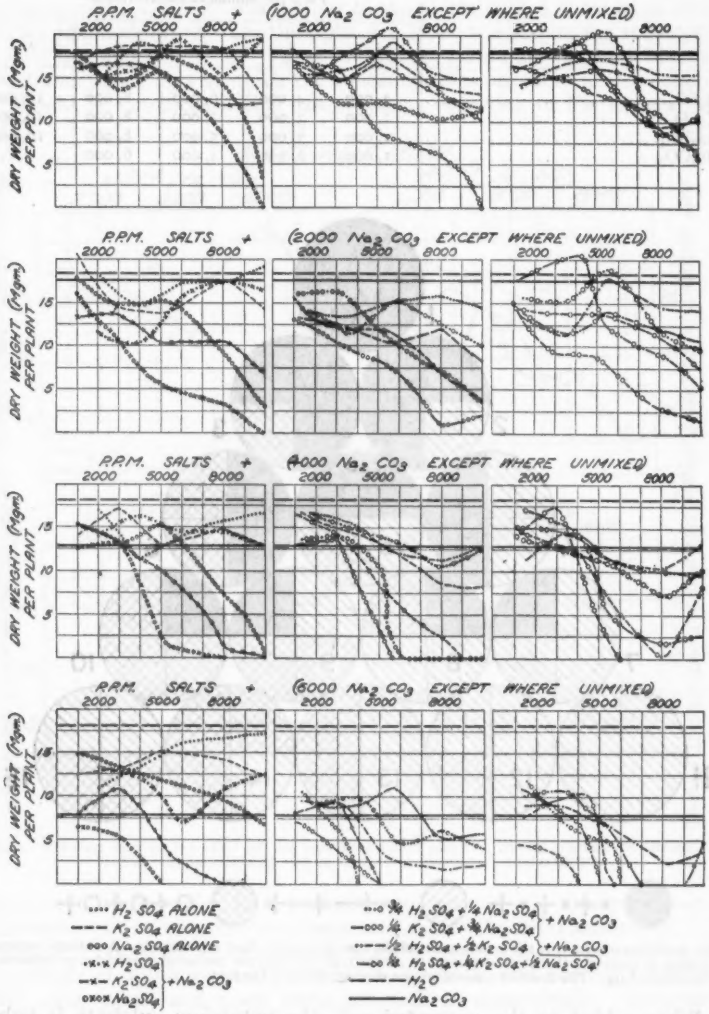
P.P.M. SALTS + (1000 Na2 CO3 EXCEPT WHERE UNMIXED)
P.P.M. SALTS + (2000 Na2 CO3 EXCEPT WHERE UNMIXED)
P.P.M. SALTS + (4000 Na2 CO3 EXCEPT WHERE UNMIXED)
P.P.M. SALTS + (8000 Na2 CO3 EXCEPT WHERE UNMIXED)
2000 5000 8000
DRY WEIGHT (Mgm.) PER PLANT
15
10
5
H2 SO4 ALONE
K2 SO4 ALONE
Na2 SO4 ALONE
H2 SO4
K2 SO4
Na2 SO4
+ Na2 CO3
3/4 H2 SO4 + 1/4 Na2 SO4
1/4 K2 SO4 + 3/4 Na2 SO4
1/2 H2 SO4 + 1/2 K2 SO4
1/3 H2 SO4 + 1/3 K2 SO4 + 1/3 Na2 SO4
H2 O
Na2 CO3

FIG. 8—Diagram showing the effect on the growth of wheat plants of adding sulphates in various proportions to Greenville loam already impregnated with sodium carbonate. The maximum concentrations of the individual sulphates are given in the text.

any toxicity, and as this condition does not obtain in the other trials it may be due to experimental error in this case. The data are not exact enough to permit quantitative relations to be drawn with certainty, but they seem to indicate that when 5,000 parts per million or more of

the sulphates are present the divergence of the curves is roughly equal to the toxicity of the carbonate.

The values for the binary and ternary mixtures given in the middle and right sections of figure 8 arrange themselves in fairly regular order

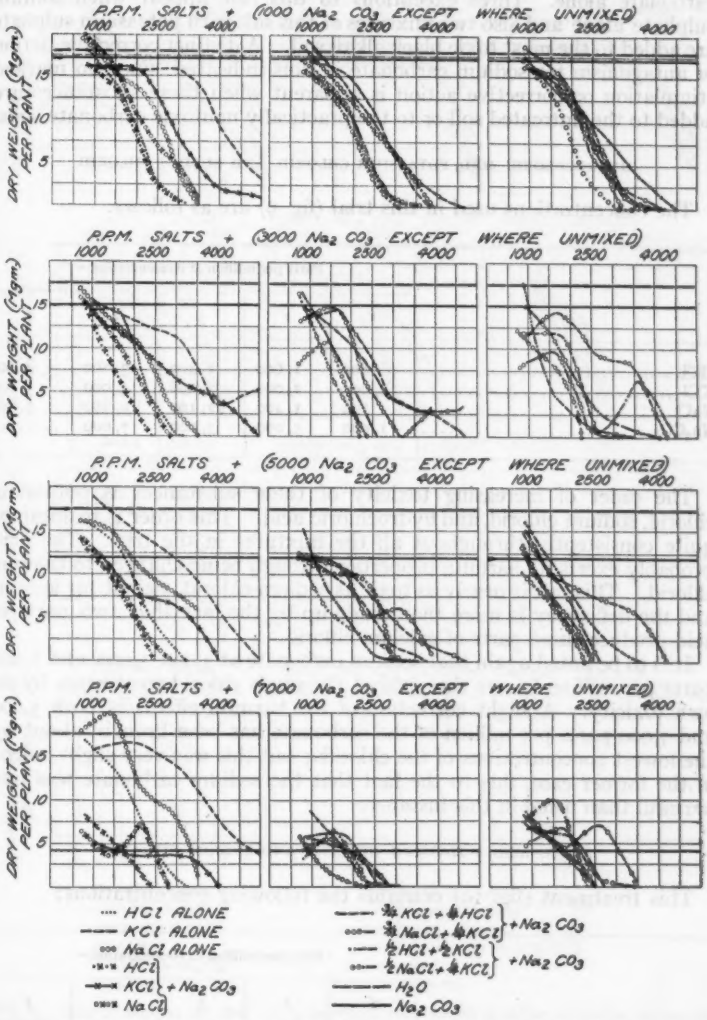

FIG. 9.—Diagram showing the effect on the growth of wheat plants of adding chlorids in various proportions to Greenville loam impregnated with sodium carbonate. The maximum concentrations of the individual chlorids are given in the text.

in accordance with the separate toxicities of the components, the individuality of these materials being essentially maintained even in the most complicated mixtures.

It should be particularly noted that the addition of nontoxic amounts of sulphates (up to 3,000 to 5,000 parts per million) to the soil containing appreciably harmful concentrations of carbonate almost invariably causes the growth of the plants to be somewhat better than with the carbonate alone. Three exceptions to this rule appear when sodium sulphate alone and also two mixtures of this salt with potassium sulphate are added to the most toxic black alkali soil. A distinct corrective action or antagonism for sodium carbonate is thus indicated, since no marked stimulation or corrective action is apparent when these substances are added to the untreated soil or to the practically nontoxic carbonate soils.

HYDROCHLORIC ACID, POTASSIUM CHLORID, AND SODIUM CHLORID

The concentrations used in this trial (fig. 9) are as follows:

	Parts per million at concentration—				
	1	2	3	4	5
HCl	800	1,600	2,400	3,200	4,000
KCl	1,000	2,000	3,000	4,000	5,000
NaCl	700	1,400	2,100	2,800	3,500
Na_2CO_3	1,000	3,000	5,000	7,000	.....

The order of increasing toxicity of these substances is potassium chlorid, sodium chlorid, and hydrochloric acid. This order is maintained quite consistently throughout all the mixtures in the trial. The acid probably exerts its harmful properties through being changed to calcium chlorid. This salt is nearly as toxic as sodium chlorid, weight for weight, and the deficiency is more than made up by the fact that two parts of acid produce three parts of calcium chlorid.

It is to be noted again that sodium carbonate at 3,000, 5,000, and 7,000 parts per million lowers the yield of the single chlorid treatments by its own toxicity. A slight correction of the harmful effects of both 5,000 and 7,000 parts per million of the carbonate has been brought about by the lowest concentrations of the chlorids; but this was very slight indeed in the former case, due to the fact that the sodium carbonate was less harmful than usual in this instance.

NITRIC ACID, POTASSIUM NITRATE, AND SODIUM NITRATE

This treatment (fig. 10) contains the following concentrations:

	Parts per million at concentration—				
	1	2	3	4	5
HNO_3	1,000	2,000	3,000	4,000	5,000
KNO_3	1,000	2,000	3,000	4,000	5,000
$NaNO_3$	1,000	2,000	3,000	4,000	5,000
Na_2CO_3	1,000	3,000	5,000	7,000	.....

The nitric acid is the most toxic material in this trial, as was the hydrochloric acid in figure 9, and the potassium salt is the least harmful. The general similarity of the nitrate and chlorid curves is evident, and

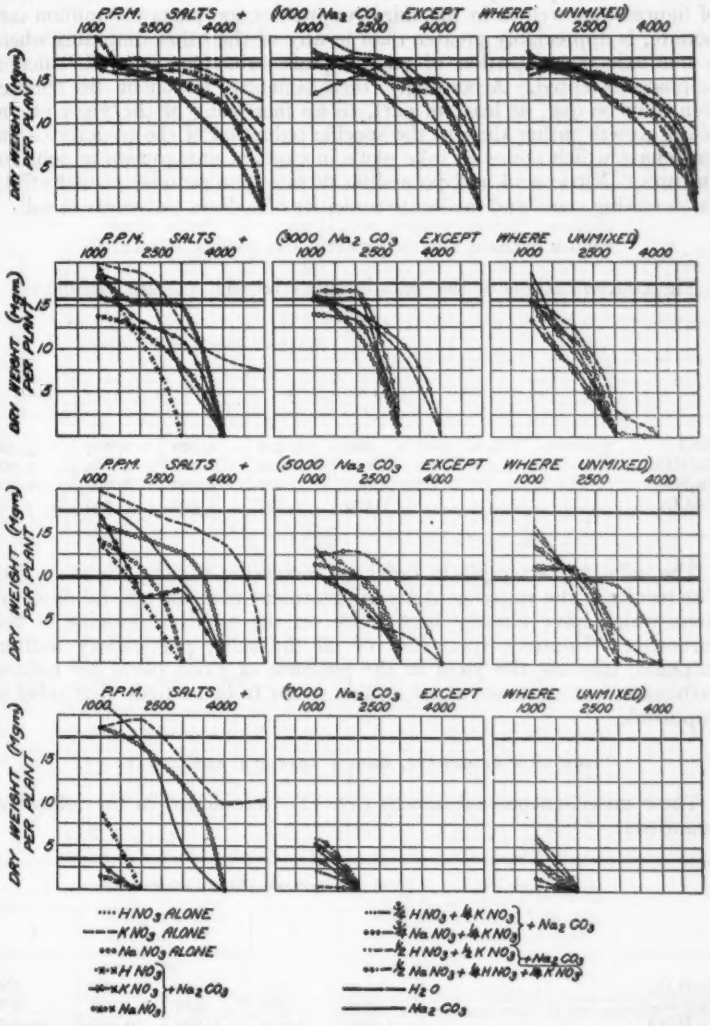

FIG. 10.—Diagram showing the effect on the growth of wheat plants of adding nitrates in various proportions to Greenville loam impregnated with sodium carbonate. The maximum concentrations of the individual nitrates are given in the text.

the same conclusions may be drawn concerning the additive toxicity of the carbonate and the corrective action of small quantities of the nitrates on this salt. In this case, however, the antagonistic action is fairly large in the third carbonate concentration and negligible in the fourth.

A special point of interest in this trial is the fact that both potassium nitrate and nitric acid are distinctly stimulating to plant growth at 1,000 parts per million. It should also be noted that their corrective action at 1,000 parts per million, as shown in the first and second rows of figure 10 as well as in the third row with 5,000 parts per million carbonate, is appreciably greater than in any of the other diagrams where a nontoxic concentration of an inorganic substance is used, calcium sulphate excepted. Accordingly, their beneficial action in this connection may be due, at least in part, to an increasing of the vigor of the plant growth rather than to the specific reduction of the toxicity of the carbonate which seems to take place in cases of antagonism in solution cultures. Nitric acid and potassium nitrate give promise of real utility in correcting small and moderate toxicities of sodium carbonate in soil.

SODIUM CHLORID, SODIUM NITRATE, AND SODIUM SULPHATE

The concentrations of the salts in this trial (fig. 11) are as follows:

	Parts per million at concentration—				
	1	2	3	4	5
$NaCl$	700	1,400	2,100	2,800	3,500
$NaNO_3$	1,000	2,000	3,000	4,000	5,000
Na_2SO_4	1,000	3,000	5,000	8,000	10,000
Na_2CO_3	1,000	3,000	5,000	7,500	.....

The salts are arranged in order of decreasing toxicity in the table. The results of the experiment are in agreement with the general observations which have already been made on the additive toxicity of the carbonate. Nontoxic quantities of all the salts, particularly sodium sulphate, increase the yield in the presence of 5,000 parts per million carbonate, but 7,500 parts per million seems to be so toxic that relief is impossible.

DISODIUM PHOSPHATE, SODIUM ARSENITE, AND BORAX

These miscellaneous substances (fig. 12) were added in the following quantities:

	Parts per million at concentration—				
	1	2	3	4	5
$Na_2B_4O_7$	140	280	420	560	700
Na_3AsO_3	194	388	582	776	970
Na_2HPO_4	1,500	3,000	4,500	6,000	7,500
Na_2CO_3	1,000	3,000	5,000	7,000	.....

The borax and arsenite are 10 times as toxic as the phosphate and they are therefore plotted in figure 12 with one-tenth the phosphate scale. Under these conditions the borax and phosphate curves are nearly coincident, but they are somewhat higher than the arsenite curve.

It is unfortunate that the initial quantity of the latter salt was so large, because no information is afforded concerning the nontoxic concentrations of sodium arsenite, except inferentially from the complex mixtures.

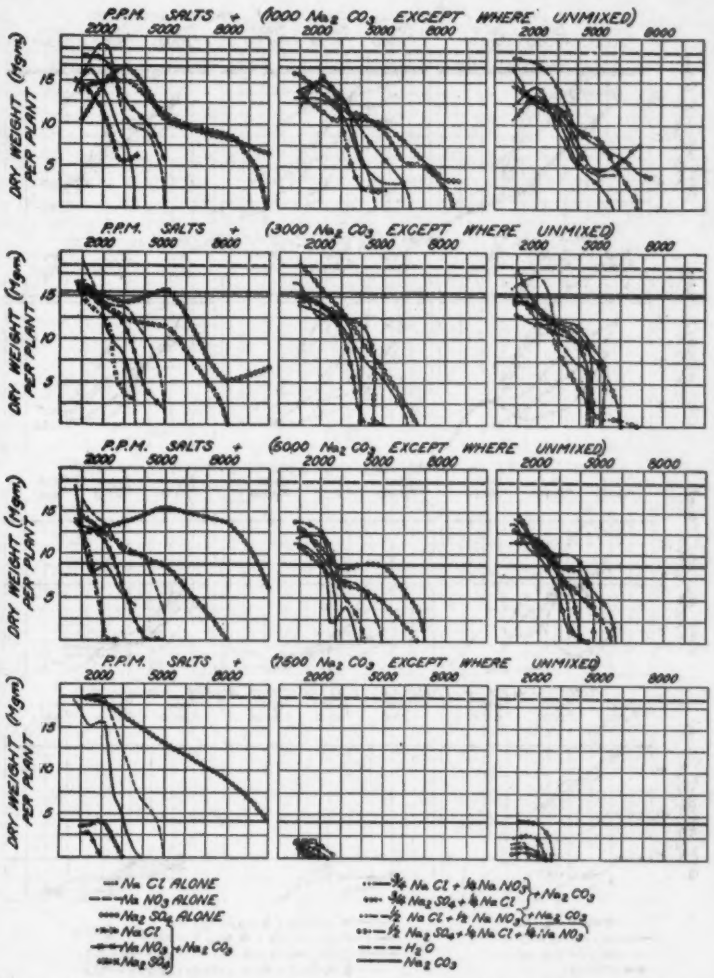
P.P.M. SALTS + (1000 Na2 CO3 EXCEPT WHERE UNMIXED)
2000 5000 8000
2000 3000 8000
2000 5000 8000
DRY WEIGHT (Hgms) PER PLANT
15
10
5
P.P.M. SALTS + (3000 Na2 CO3 EXCEPT WHERE UNMIXED)
P.P.M. SALTS + (5000 Na2 CO3 EXCEPT WHERE UNMIXED)
P.P.M. SALTS + (7500 Na2 CO3 EXCEPT WHERE UNMIXED)
Na Cl ALONE
Na NO3 ALONE
Na2 SO4 ALONE
Na Cl + Na2 CO3
Na NO3 + Na2 CO3
Na2 SO4 + Na2 CO3
3/4 Na Cl + 1/4 Na NO3 + Na2 CO3
3/4 Na2 SO4 + 1/4 Na Cl + Na2 CO3
1/2 Na Cl + 1/2 Na NO3 + Na2 CO3
1/2 Na2 SO4 + 1/4 Na Cl + 1/4 Na NO3
H2 O
Na2 CO3

FIG. 11.—Diagram showing the effect on the growth of wheat plants of adding sodium salts to Greenville loam impregnated with sodium carbonate. The maximum concentrations of the individual salts are given in the text.

The usual regular arrangement of the mixture curves and also the additive toxicity of the carbonate is evident in the diagram. No corrective action on black alkali, however, is shown in this experiment.

The physiological action of the borax and arsenite on the wheat plants was so striking and so characteristic that it should be mentioned here. Borax caused the leaves of the plants to remain closed up around

the stock, which then twisted itself into curious shapes, at the same time exhibiting a distinct chlorotic condition. The intensity of these symptoms varied with the concentration of the borax. They were dis-

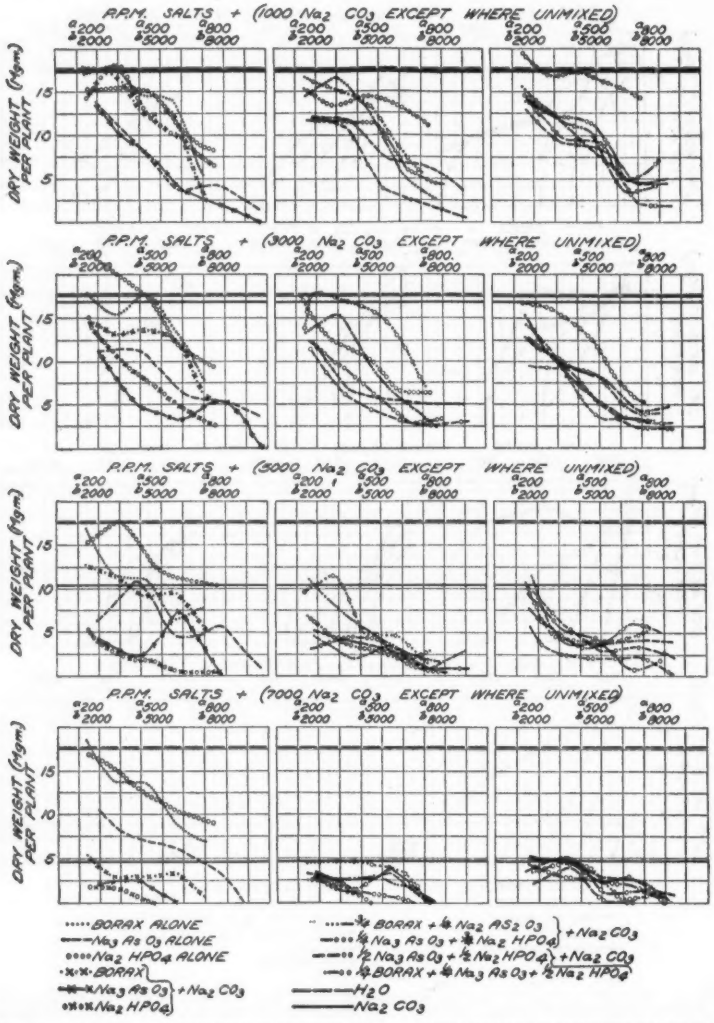
P.P.M. SALTS + (1000 Na2 CO3 EXCEPT WHERE UNMIXED)
P.P.M. SALTS + (3000 Na2 CO3 EXCEPT WHERE UNMIXED)
P.P.M. SALTS + (5000 Na2 CO3 EXCEPT WHERE UNMIXED)
P.P.M. SALTS + (7000 Na2 CO3 EXCEPT WHERE UNMIXED)
DRY WEIGHT (Mgm.) PER PLANT
BORAX ALONE
Na3 As O3 ALONE
Na2 HPO4 ALONE
BORAX
Na3 As O3
Na2 HPO4
+ Na2 CO3
H2O
Na2 CO3

FIG. 12.—Diagram showing the effect on the growth of wheat plants of adding miscellaneous sodium salts in various proportions to Greenville loam impregnated with sodium carbonate. The maximum concentrations of the individual salts are given in the text.

[a] This reading applies for a sodium arsenite or borax mixture or where used alone.

[b] This reading applies for sodium phosphate mixtures or where used alone.

cernible with as little as 70 parts per million, though at the lowest concentrations they tended to disappear as the plant grew. The arsenical poisoning was characterized by a stunted growth, straight, leafless stock, and a dark, unhealthy color.

CALCIUM SULPHATE, SULPHUR, AND MANURE

For the sake of completeness three well-known black alkali amendments were included in the experiment. Since none of them were toxic, the concentrations were made to range beyond the limits set by agricultural practice:

	Parts per million at concentration—				
	1	2	3	4	5
$CaSO_4$	1,000	3,000	6,000	10,000	20,000
Sulphur	200	1,000	3,000	6,000	10,000
Barnyard manure	2,000	5,000	10,000	15,000	20,000
Na_2CO_3	1,000	2,000	5,000	7,000	.....

The materials were mixed with the soil in a dry, powdered condition before the water was added. With the larger quantities of manure the soil was somewhat deficient in moisture because of the larger absorptive power of this treatment for water. The results for manure can not, therefore, be taken as final, since under other moisture conditions a different behavior might be expected.

The experiment (fig. 13) shows that all three added substances are somewhat stimulating alone and that all have the power of counteracting partially the toxicity of sodium carbonate. This corrective action is accomplished in the lower carbonate treatments about equally well by all three materials, but with sodium carbonate at 7,500 parts per million calcium sulphate is the most effective and even seems to be a necessary component of the amending mixture. It should be noted that curves with 7,500 parts per million sodium carbonate represent average values for two entirely independent but closely concordant experiments.

SUMMARY

(1) The toxicity relations for wheat of certain alkali salts alone and in combination with each other have been investigated with special reference to the alleviation of black alkali trouble.

(2) It has been shown that when a given concentration of sodium carbonate in sand is treated with an equal quantity of calcium sulphate or sulphuric acid, an appreciable lowering of the carbonate's toxicity is evident. This relation, however, is not so manifest in heavier soils. Sodium sulphate increases the toxicity of the carbonate under the same circumstances.

(3) Either sodium chlorid or sodium nitrate could be substituted for sodium carbonate in the arrangement described above without changing the results. No antagonism between these salts and the sulphates could be detected in Greenville loam.

(4) During 1921 each one of four different concentrations of sodium carbonate, already incorporated in the Greenville soil, has been treated with five concentrations of the following substances, in groups of three:

(1) Sulphuric acid, potassium sulphate, and sodium sulphate.
(2) Hydrochloric acid, potassium chlorid, and sodium chlorid.
(3) Nitric acid, potassium nitrate, and sodium nitrate.
(4) Sodium chlorid, sodium sulphate, and sodium nitrate.
(5) Sodium arsenite, sodium phosphate, and borax.
(6) Calcium sulphate, sulphur, and barnyard manure.

(5) The toxicities of all the substances in the first five groups are increased by the specific toxicity of sodium carbonate when they are added to soil already impregnated with this salt.

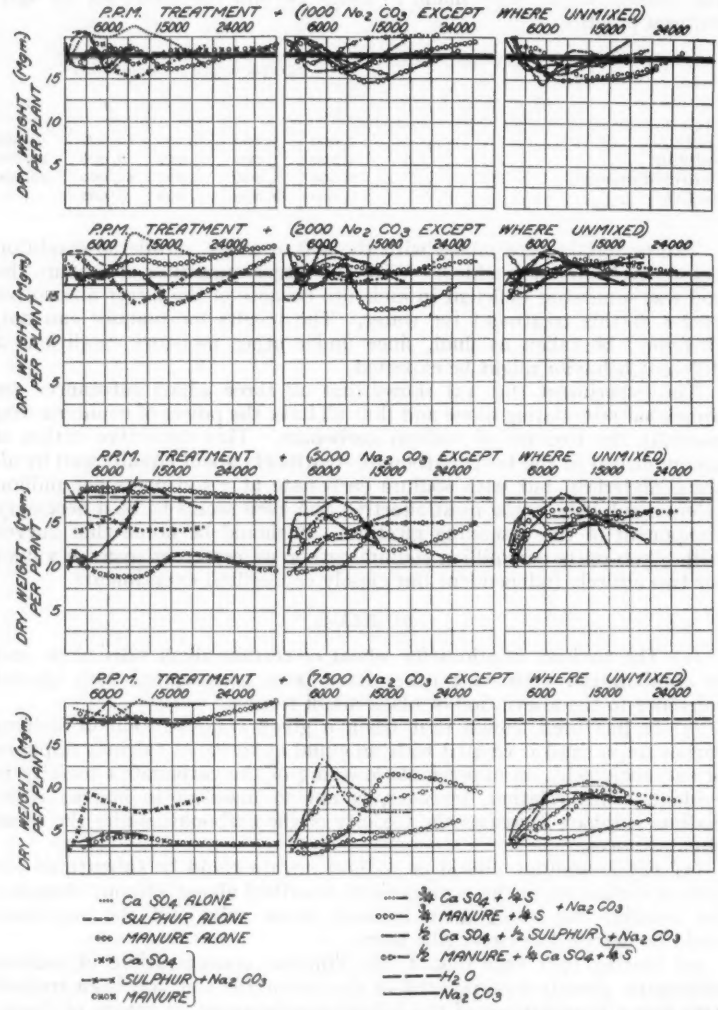

FIG. 13.—Diagram showing the effect on the growth of wheat plants of adding manure, sulphur, and gypsum in various proportions to Greenville loam impregnated with sodium carbonate. The maximum concentrations of the individual added substances are given in the text.

(6) Nontoxic quantities of all the substances in the first four groups have the power of correcting, to some extent at least, the harmful effects of moderate, and in some cases of fairly high, concentrations of sodium carbonate. The salts in the fifth group do not exhibit this property at

all. This phenomenon may be due in part at least to specific stimulation of plant growth by these substances rather than to any antagonistic action on the sodium carbonate which would lower the toxicity of that salt as is frequently observed in solution culture.

(7) Potassium nitrate and nitric acid are both distinctly stimulating to plant growth at 1,000 parts per million, and the addition of this quantity of these materials to soil containing 5,000 parts per million sodium carbonate or less was particularly beneficial.

(8) Under the conditions of the experiment, barnyard manure was an effective amendment for soil containing 2,000 parts per million sodium carbonate, but its corrective power for more toxic concentrations of black alkali was much less evident, probably because the optimum moisture content of the soil was not maintained when the larger amounts of manure were added.

(9) Calcium sulphate, alone and in conjunction with sulphur and manure, is the most effective corrective that was tried, particularly on the more toxic black alkali soils.

(10) The curious physiological effects of borax and sodium arsenite on the wheat plants have been noted.

(11) While the data herein presented throw some light on the antagonistic action of various alkali salts, it is evident that the problems of coping with black alkali is far from being solved.

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IDENTIFICATION OF CERTAIN SPECIES OF FUSARIUM ISOLATED FROM POTATO TUBERS IN MONTANA [1]

By H. E. MORRIS, *Assistant Botanist and Bacteriologist*, and GRACE B. NUTTING, *Assistant in Botany and Bacteriology, Montana Agricultural Experiment Station*

INTRODUCTION

The econon ic importance to this State of Fusarium wilt and various storage and field rots of potatoes has been recognized for several years. Between 1914 and 1918 isolations were made from affected tubers until nearly 100 cultures had been obtained. These isolations have been cultured on artificial media, being transferred about twice a year and kept in a refrigerator at about 10° C.

The purpose of this paper is to record the taxonomic work on these various Fusarium cultures. No especial endeavor was made to connect any of these species with an ascigeral stage, and in all our work no culture showed any form of growth suggesting such a stage.

SOURCE OF MATERIAL

The tubers from which the isolations were made came from many different localities in the State. In fact, practically all sections except the extreme northwestern are represented. The exact source of each culture will be found in Table I. Of the 97 original isolations only 70 were used.

There is little doubt that dryrot and Fusarium wilt are of large economic importance to all potato growers in the State. In 1917, this Station reported in the plant disease survey a loss of 4 per cent in the potato crop due to wilt and a loss of 3 per cent due to dryrot. The year 1917 is considered a normal one. These figures therefore represent the average yearly loss due to potato diseases caused by species of Fusarium. This loss is fairly evenly distributed over the State.

LITERATURE REVIEWED

A great deal of work has been done and a number of papers have been written on Fusarium troubles of potatoes, but the viewpoint in nearly all of this work is economic. However, in reporting on the economic phase many of the authors include some taxonomic data, and for this reason the following reviews are given. Occasional comment on individual papers has been made, but this has not been done consistently throughout. For convenience the various papers are taken up in chronological order.

Smith and Swingle (*19*) [2] found that there was always present in the darkened vascular bundles of tubers affected with wilt, which had not at that time been separated from dryrot, a fungus which on culturing they found to be a species of Fusarium. In order to determine the characters of this fungus they grew it on about 40 different media and under various temperature conditions. The bulletin gives in detail the results of these

[1] Accepted for publication July 24, 1922.
[2] Reference is made by number (italic) to "Literature cited," p. 363–364.

studies. To this organism the authors applied the name *Fusarium oxysporum* Schlechtendal because they felt it "not at all certain" that the various names given to species of Fusarium growing on potatoes really stood for distinct forms. They therefore considered them as synonyms and used the earliest available name. This paper was the pioneer for Fusarium work in this country, and in addition to the careful work done, mycologists and pathologists are greatly indebted to its authors for the impetus given to the study of potato Fusaria, both in this country and abroad.

In 1910 Appel and Wollenweber (*2*) published a basis for a monograph of the genus Fusarium Link. This paper was the first to give a comparatively exhaustive treatment of the species of Fusarium. It is divided into two parts. In the first part the following subjects are discussed in detail: Methods, including media, inocula, nutrients, light, temperature, color standards, variation in forms appearing in the cultures, mycelium, etc., lack of distinction between microconidia, and macroconidia and characters which constitute a "normal" culture or "normal" spore. These authors give a description of the genus Fusarium, discuss its relationships, and list its synonomy.

The second part relates entirely to taxonomy. Thirteen known species are described with the greatest care and detail, each description being the record of a research problem in itself. This paper is, without doubt, the most fundamental in the literature on Fusarium. It is not here reviewed in proportion to its worth, because our experimental work was not directly influenced by it. The paper was published in Germany 10 years ago and did not include many of the common American species. Therefore, it was not well fitted for our identification work. The paper contains a very good bibliography of the Fusarium problem.

In 1912 Jamieson and Wollenweber (*9*) described the symptoms of a disease causing dryrot of potato tubers, first noted on tubers sent from Spokane, Wash., in February, 1910. To the causal organism of this disease Wollenweber gave the name *Fusarium trichotheciodes*, placing it in the Discolor group. Inoculation experiments proved the pathogenicity of this organism in producing the characteristic dryrot of tubers.

In 1912 Wilcox, Link, and Pool (*20*) published a research bulletin in which they described a rot which is practically identical with that described by Jamieson and Wollenweber (*9*), but due to the fact that the two papers were published so close together and that the investigational work was being carried on simultaneously and independently, these authors gave to the casual organism the name *Fusarium tuberivorum* Wilc. and Link, which we know now to be a synonym of *F. trichothecioides* Wollenw. Wilcox, Link, and Pool's paper is of particular value to our work because of its emphasis on taxonomy.

This paper gives the history and distribution of the dryrot of tubers and the economic importance and symptoms of the particular dryrot under discussion. The authors give a résumé of the genus Fusarium and allied genera from 1809 to date, concluding with Appel and Wollenweber's description of it.

Details are given concerning the technic for studying the fungus, such as temperature, light, single-spore isolation, and color standard; and a discussion is made of macroscopic characters, mycelium, influence of temperature, influence of humidity, color characteristics, conidiophores, chlamydospores, spore measurements, and spore septations. Considerable space is given to the last-mentioned topic. The importance of

various conditions which might influence the number of septations is discussed, and the results of actual investigations are recorded in detail.

In summarizing the taxonomic portion of the paper the authors endeavor to find a logical place for their species, and after several suggestions and comparisons they leave it unplaced, but give a detailed description of the organism. Some pathological studies are discussed with emphasis on the mode of infection, resistance, and susceptibility of varieties and methods of control.

Wollenweber (*21*), studying the genus Fusarium taxonomically, came to a few definite conclusions: The stroma as a taxonomic character in species determination is unreliable. A pure culture method which gives the normal stages is necessary, and the culture media recommended was steamed stems of trees, shrubs, or herbaceous plants for conidia and chlamydospore production and rice, potato tubers, and other starchy media for secondary characters, such as color, extra large sclerotia, and stromata. He is not sure that Fusarium is an obligiate conidial stage of an ascomycete. He puts much emphasis on the importance of the proof of pathogenicity of the organisms, and in his discussions of each species he indicates the kind of parasite.

Wollenweber was the first to assemble into sections species of Fusarium having related characters. He considered a uniform shape of conidia the most important of the characters on which the division could be based. The sections which he described in this paper have been used in all taxonomic work with this genus since that time. Other sections, of course, have been added, but the original sections have for the most part never needed to be amended.

The 20 species of Fusarium described, of which only 3 are new, are grouped under the sections, practically all of which include wound parasites capable of destroying parenchymatous tissue, except the first, which includes vascular parasites only. Wollenweber's sections in their order are: Elegans, Martiella, Discolor, Gibbosum, Roseum, and Ventricosum.

Later Wollenweber (*22*), in morphological and pathological study of the divisions of fungi having cylindrical and crescent-shaped conidia, states definitely that fungi with cylindrical septate conidia fall outside the genus Fusarium, and belong, when the perfect form is known, to the genera Nectria, Hypomyces, and Mycosphaerella, and, when the perfect form is not known, to Cylindrocarpon when chlamydospores are absent and to Ramularia when they are present.

Lewis (*11*), working in Maine, carried on comparative studies with some 40 different cultures of Fusarium isolated from various hosts, including 7 isolated from potatoes. He noted how the growth of the cultures was effected by different media, different quantities of acid and alkali, and different temperatures. He tested for gas in fermentation tubes, but obtained negative results only. Tests of pathogenicity were carried on with all of the cultures, and considerable cross inoculation work was done. He made no attempt to identify the species, because the published descriptions were so incomplete as to make critical comparisons with them impossible. However, Wollenweber was in the United States at that time, and Lewis sent his cultures to Wollenweber, who identified most of them. His results were added as an appendix to the bulletin.

Four of these species—*Fusarium poae, F. helianthi, F. conglutinans,* and *F. pirium*—do not later appear in the literature as occurring on potato. Sherbakoff (*18*) says of the first two that they are closely

related to *F. sporotrichioides* n. sp. and belong to the section Sporotrichiella; the third species belongs to section Elegans and is closely related to *F. orthoceras;* and the fourth may belong to section Arthrosoriella. Sherbakoff does not recognize any of these species but disposes of them all in saying:

> No technical description, except results of inoculations for potatoes, always negative, and certain characters of color and of colony growth, is given, and thus a proper identification is rendered impracticable.

Harter and Field (7) from the results of their work on the stemrot of the sweet potato concluded, as Appel and Wollenweber (*2*), that the type of inoculum—mycelium or spores—has a marked influence on the culture. They also proved the pathogenicity of *Fusarium hyperoxysporum* Wr. and *F. batatis* Wr. for the sweet potato and obtained negative results in their inoculation experiments with *F. oxysporum* Schlecht., *F. orthoceras* Ap. and Wr., *F. caudatum* Wr., and *F. radicicola* Wr. on the same host.

Wollenweber (*23*) discussing the species of Fusarium occurring on sweet potatoes, points out the necessity of agreeing on the criteria by which a "normal" culture may be known, to avoid wide discrepancies in describing what is in reality the same species. He describes 11 species of Fusarium, two species of Hypomyces and one of Gibberella occurring on sweet potatoes. This included all the species of Fusarium then known to occur on sweet potatoes. Under each species is given a "diagnosis" or description of the type culture, habitat, and a general discussion of its history and relationships. In the case of new species, of which there are 6, the relationships are taken up with a great deal of care.

A descriptive key to all these species is included based upon the characteristics of pure cultures grown in daylight. Regarding the key the author makes the following comment:

> This key might have been based entirely upon the morphological characters and curvature of the conidia but since the color reactions offer a simpler, though less trustworthy means of identification, they have been employed. This key, therefore, should be regarded only as an aid in identification, not as a guide to the morphology, which has been discussed in the diagnosis and illustrated in detail in the illustrations.

Sherbakoff (*18*) realizing the chaotic condition of the genus Fusarium, especially those species occurring on potato, conducted a research problem to discover on what basis the American species as well as those discussed by Wollenweber could best be separated.

In general, Sherbakoff verified the principles and the results of Appel and Wollenweber's (*2*) work in Germany. He believed, however, disagreeing with Appel and Wollenweber, that there should be a distinction drawn between microconidia and macroconidia, and that the presence or absence of the microconidia may be used in distinguishing species. He also disagrees with Appel and Wollenweber in believing that species can be distinguished—

> when grown on almost any medium, including artificial media, provided that the medium is not extremely poor or rich in food materials, and also provided that the moisture supply in the medium is well regulated.

Sherbakoff found no coremia nor typical pionnotal form of fructification as did Appel and Wollenweber.

The author outlined the scope of the work, discussed the source of material and methods of isolation, culture media, effect of light and temperature, variability in the species of Fusarium, relative taxonomic

importance of different characters, defined certain forms of fructification, reviewed the genus Fusarium, and explained the difficulties of identifying species of Fusarium with previously described species because of the scarcity of taxonomic detail in the descriptions. The greater part of the memoir is taken up by the description of sections, genera, species, and varieties. He listed 20 previously described species, including parasitic and nonparasitic forms—all that were known to occur on potatoes—and 41 new species and varieties. These 61 forms he distributed under Wollenweber's eight sections and three additional ones that he himself originated and defined. Three species of Ramularia, a genus closely related to Fusarium and also occurring on potatoes, are included. There are drawings of practically all species and varieties, showing conidia, conidiophores, and occasionally mycelium. Spore measurements are given for spores grown on various culture media.

Based on the ideas of Appel and Wollenweber, Sherbakoff (*18*) worked out a dichotomous key for the species of Fusarium described, which, while the best yet published, is far from perfect. Imperfections in keys are rather inevitable until methods used in the identification of the species are better standardized. Until standard methods are adopted, the boundaries of species can not be closely enough defined to prevent investigators from introducing numerous varieties, separating one from another on minor characters that are not stable under all conditions.

In the key, Sherbakoff uses septations and shape of conidia most often as differential characters. Presence or absence of microconidia, chlamydospores, sclerotia, sporodochia, type of fructification, and color and type of conidiophores are also used. Difficulty with the key arises most often in the case of varieties. Individual difficulties of this sort will be noted later. Confusion often arises from the misuse of the terms macroconidia and microconidia, but this may be due to typographical errors.

C. W. Carpenter (*4*), in a paper on tuber-rots caused by species of Fusarium, includes a section on taxonomy which gives the description of eight species. Among these one is new, *F. eumartii*, which falls into the section Marteilla.

Link (*13*) shows some very interesting results from physiological studies on *Fusarium oxysporum* Schlect and *F. trichothecioides* Wr. Comparisons of the two species are given to show temperature relations, growth, habit, and food requirements and pathogenicity to tubers and growing plants.

Pratt (*15*), in a paper on control of powdery dryrot caused by *Fusarium trichothecioides* Wr., concludes that this species is of the highest economic importance of all of the Fusarium species in the irrigated portion of the West, and in another paper (*14*) notes that *F. radicicola* Wr. is rather common in desert soils.

Hawkins (*8*), in studying the effect of certain species of Fusarium on the composition of the potato tuber, found that *Fusarium oxysporum* and *F. radicicola* secrete sucrass, maltase, xylase, and diastase. The last-mentioned enzym is apparently incapable of acting on the ungelatinized potato starch. The purpose of the study was to find out what constituents of the potato are most easily destroyed by the fungus and what compounds can not be utilized by it either in respiration or in building its own tissues. Their results are not conclusive as to whether kind or quantity of secretion is in the least specific, as only three organisms were used in their experiments, but this article introduces an interesting phase of the Fusarium problem.

Pratt (*16*), in studying the relation between soil fungi and diseases of the Irish potato in southern Idaho, isolated among many other fungi 14 species of Fusarium. Five of the strains isolated "apparently differed from all species heretofore described," and Pratt, therefore, named them, giving in this publication the original descriptions, which include habitat, cultural characteristics, spore shape, septations, and size. Septations and size are given of spores grown on various media for different ages.

The new species described are: Section Gibbosum, *Fusarium lanceolatum;* Section Elegans, *F. elegantum* and *F. Idahoanum;* Section Discolor, *F. aridum* and *F. nigrum.*

The other nine species were identified, but in only one case, that where Pratt's culture showed some differences from the "authentic culture" which he used for comparison, are there any taxonomic notes.

Bisby (*3*) in his studies on Fusarium diseases in Minnesota notes that *Fusarium oxysporum* and *F. discolor* var. *sulphureum* are of large economic importance in Minnesota. His results with certain temperatures and media in studying these diseases are of interest to use, but otherwise the bulletin is strictly economic in its outlook.

Edson and Shapovalov (*5*) made a careful study of the relations of growth of certain species of Fusarium to temperature. The species they used in the studies were: *Fusarium discolor*, var. sulphureum (Schlect) Ap. and Wollenw., *F. eumartii* Carp., *F. oxysporum* Schlect, *F. radicicola* Wollenw., *F. trichothecioides* Wollenw.

Two species of Verticillium were also used. For each of these species they made nine plate cultures and incubated them at nine different temperatures from 1° C. up to 40° C. at 5° intervals, taking readings of the size of the colony at the end of each 24 hours. The results, aside from aiding in control determinations, proved to the authors that temperature tests in certain cases may serve as a useful supplementary method for the identification of fungi exhibiting contrasting thermal relations.

THE GENUS FUSARIUM LINK.

The genus Fusarium is classified according to Engler and Prantl Naturliche Pflanzenfamilien (*6*) as belonging to the section Mucediacae Pharagmosporae of the family Tuberculariaceae of the order Hyphomycetes of that heterogenous class known as the Fungi Imperfecti. It is, consequently, a form genus, and already the ascigerous stage of a number of its species has been found. A few of these are *Nectria solani* (Ren. and Bert.), which has been reported as the ascigerous stage of *Fusarium solani; Nectria graminicola* B and N., as the ascigerous stage of *F. nivale;* and *Gibberella saubinetii* (Durieu and Mont.) Sacc. to which species *F. tulmorum, F. avenaceum, F. hordei*, and *F. heterosporum* have all been referred. It seems very probable that more and more species of this genus will be connected with genera of the Ascomycetes, though as Wollenweber states (*21*)—

> We are still far from having conclusive proof of the widely recognized theory that *Fusarium* is the obligate conidial stage of Ascomycetes.

The genus Fusarium was described in 1809 by Link (*12*), together with the allied genera Fusidium, Fusisporium, and Atractium. Later Link dropped one or the other or combined them in various ways. Schlectendahl, Corda, Fries, and Saccardo worked on this group of organisms and

classified them in various ways, but they all recognized the imperfections of the classification. In their monograph Appel and Wollenweber (*2*) have established the boundaries of the genus Fusarium, using Atractium Link, Fusidium Link, Fusisporium Link, Selenosporium Corda, Fusoma Corda, and Pionnotes Fries, either in toto or in part, as synonyms.

The synonomy of the genus Fusarium given by Appel and Wollenweber (*2*) is quoted below, and a translation is given of their description and notes.

Synonomy:
Atractium Link pr. p. *in* Mag. Ges. Nat. Freunde III, S. 10 (1809).
Fusidium Link pr. p. *in* Mag. Ges. Nat. Freunde VII, S. 31 (1816).
Fusidium Link pr. p. *in* Spec. Plant 11. S. 96 (1825).
Fusisporium Link *in* Spec. Plant 1, S. 30 (1824).
Selenosporium Corda Icon. I. S. 7 (1837).
Fusoma Corda Icon. I. S. 7 (1837).
Pionnotes Fries Sum. Veg. Scand., S. 481 (1849) Sacc. Syll. IV, S. 725.

Conidia more or less polar, mostly dorsiventral, seldom distinctly round (radiär), more or less curved; when ripe usually septated; more or less colored when in masses; borne one after another in the same spot, but not connected in chains on the end of simple or branched septate conidiophores which appear spread out between the hyphae or joined as they are in coremia, or grouped together in sporodochia. Conidia spread out in a powdery form between the hyphae or tubercular-like on a limited gelatinous sporodochia, a slimy layer or occasionally as pionnotes without definite boundaries.

Chlamydosphores, oval or pear shaped, single or in bunches, in chains or bunched up, remaining joined for some time, terminal or intercalary, not more than one borne in the same place. The chlamydophore is not very different from the conidiophore, and it has no distinctive color. It never gathers in gelatinous layers.

Hyphae septate, variously branched epi- and endo-phytic, occurring sparingly or in great quantity, either isolated or together, curly or thick, partly like coremia, or especially like a stroma to plectychymatic form with definite shape or without definite shape, more often similar to an even growth all over, limited or spread out, often closed up together on the inside, occasionally building up brightly colored mycelium.

Note that it is undecided whether species that do not have septate conidia should be kept separate from the genus or be placed in a subgenus *Fusamen* according to Saccardo (*17*); but there is no question about those which have a tendency toward septation as *F. orthoceras*. It is also undecided in what order of importance the characters should be taken. The choice is between septations, dorsiventrality, polarity and the curve of the long axis of the conidia. It is very questionable whether *Fusarium* should be placed under Leptosporium as in Saccardo, and nothing but the study of the different forms can decide the boundaries of the genus. Concerning the color of the conidia masses it can be said that black does not appear normally, neither does black mycelium. Light orange and ochre colors predominate in the conidia. The mycelium also has yellow, red and blue. The term sclerotium as used in *Fusarium* is disputable. Researches have not shown that the term sclerotia was justifiable for the plectychymatic structures found.

In 1913 Wollenweber (*22*) excluded from the genus all species having septocylindrical conidia. In bringing this genus to date, therefore, this fact should be incorporated. Sherbakoff (*18*) describes the genus concisely as follows:

Hyphomycetes, with from hyaline to bright, but never plain gray nor black, conidia and mycelium; conidia sickle-shaped, septate (usually 3 or more septate), apically pointed, mostly pedicillate, not appendiculate, noncatenulate; conidia scattered over substratum, in pseudopionnotes or in sporodochia, the latter without or with from flat to wart-like plectenchymic substratum, and always without any differentiated enclosing or surrounding structures; conidiophores from simple to irregularly verticillate.

ISOLATION OF CULTURES

The original cultures were all obtained from infected tubers by the following method:

The infected tubers were thoroughly washed, dipped into a 1 to 1,000 solution of mercuric chlorid, and cut open. In order not to contaminate the infected parts of the tuber, the healthy portion was cut almost to the edge of the discolored portion and the tuber then broken open, care being taken that nothing should touch the advancing margin of the fungus. A piece of discolored tissue was taken from the advancing margin and put into a tube of melted agar, which was then poured into a Petri dish and incubated at room temperature. Six to 10 plates were made from a single tuber. The plates were watched carefully to be sure that a pure culture was the result of the isolation. When this was assured a transfer was made to agar slants, and these were kept as stocks. It was surprising how very few contaminations appeared in the plates. Each culture was numbered, and all notes, including source, were kept under this number.

SINGLE-SPORE ISOLATIONS

In 1916 when detail work with these cultures was anticipated it seemed best to take every precaution to be assured of pure cultures, for a mixed culture of two or more species of Fusarium could easily pass unnoticed. Therefore, a single-spore isolation was made from each culture in the following manner:

A sterile platinum needle was used to transfer spores from the stock culture to tube 1, which contained 5 cc. of distilled sterile water. From this tube a series of five dilutions was made into tubes, each containing 5 cc. of sterile distilled water. Three 3-mm. loops of material were taken from tube 1 and put into tube 2; three 3-mm. loops of material were taken from tube 2 and put into tube 3; and three 3-mm. loops of material were taken from tube 3 and put into tube 4. Tubes of standard beef agar were melted and poured into Petri dishes and allowed to cool. From each dilution tube ½ cc. of material was made to flow over the surface of the plated agar. Any excess material was drained off. The plates were allowed to incubate at room temperature for about 16 hours, when they were searched with a microscope for germinating spores. All microscopic examination was made through the bottom of the inverted Petri dish.

Fusarium spores are for the most part hyaline and to locate them on a plate of clear agar is very difficult. The scheme was devised of sprinkling a few sterile spores of *Tilletia foetans* of wheat over the surface of the agar with a tiny sterile spatula. These spores were easy to locate and gave the plane of focus in which the Fusarium spores could be found. The use of the sterile smut spores proves to be a great timesaver.

Usually the search for spores began with the more heavily sown plates, down through the more dilute ones until a spore was found which was sufficiently isolated so that it alone could be removed. The position of this spore was marked by a ring of India ink on the glass and it was then cut out by means of a stiff platinum cylinder illustrated in Phytopathology (*10*) and placed on the upper end of an agar slant. It was carefully watched by means of the microscope to be sure that all growth came from the spore and not from a piece of mycelium from the edge of the piece of agar, as often happened when the germinating spores were not sufficiently isolated. When it was assured that all growth came

from the spore it was considered pure and the culture was kept as a stock. If there was any question as to the source of the growth the culture was discarded. Sometimes a number of attempts had to be made before the stock culture was obtained.

COMPARATIVE CULTURAL STUDIES

Since the purpose of our work was the identification of the species of Fusarium that we had found occurring on potatoes in Montana, we began by comparing the cultural characteristics of each isolation product, hoping to be able to group together those which belonged to one species so that we might eliminate unnecessary duplication with cultures of the same species. This hope was realized only in one group. After a very few trials with different media and at different ages, one group, containing about 20 per cent of all the cultures, separated itself out very constantly. Its growth was so characteristic that we then believed and have since proved that it was *Fusarium trichothecioides* Wr. There were other groups, some seven of them, each containing from 2 to 10 cultures; but the identity of the members of the groups was not sufficiently striking to warrant leaving any of them out of further studies. However, these groupings aided materially when actual identification work began. Seventeen series of cultures have been studied. A series consisted of all the transfers made at the same time and incubated under similar conditions for the purpose of comparative studies. At least four sets of individual notes were taken on each series, so as to bring in the influence of age on the characters.

The characters emphasized in these notes were color, amount, and nature of the growth of mycelium, absence or presence and color of pseudopionnotes or sporodochia. All color determinations were based on Ridgway's "Color Standards." [3]

In practically all the series the cultures were inoculated in triplicate, so that when variations arose between cultures inoculated from the same stock culture a decision as to which was the more normal could be made.

As a preliminary to note taking, those cultures which had sufficient similar cultural characters to suggest identity were put into groups. Each group was designated by a letter, and these were placed in a table in parallel vertical lines, a column for each note taking, so that gross group comparisons could easily be made. Notes on each group were made in detail at the end of the table. If a culture showed only a slight variance from a group it was put into the group, but with special additional notes, and was designated in the table by the group letter with a subnumber. In so far as possible the same letters were used throughout for the same group. After a few sets of notes were taken one or two cultures which seemed most typical of a group were chosen as type cultures, and these numbers were given the same letter each time and served as the nucleus for the group represented by that letter.

Some few cultures, the number varying with the medium on which they were grown, did not develop any distinctive microscopic characters; others developed distinctive characters on certain media; and occasionally the same culture when on one medium was placed in a certain group, while on another medium it would be placed in a different group, or, as

[3] RIDGWAY, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 col. pl. Washington, D. C. 1912.

often happened, would seem to fit none of the groups. These refractory cultures caused considerable trouble, and some of them have not yet been identified.

MICROSCOPIC STUDIES

USE OF PHOTOMICROGRAPHS

The microscopic studies that are most extensively used in classifying Fusaria are the size, shape, and septation of the spores. In view of the fact that different kinds of spores are produced and that in each kind there is much individual variation, the problem becomes in some cases rather complex.

In a goodly portion of the microscopic studies photomicrographs were used. The spores were mounted in water containing a small quantity of dilute Myer's flagella stain. The cover glass was blotted with a coarse filtered paper, pressed down, and sealed with paraffin to prevent evaporation and consequent movement of the spores. The pictures were taken soon after the slides were made, for the mounts gradually dried out. If they were put into a moist chamber they could sometimes be kept intact for 24 hours or more.

The Leitz horizontal photomicrographic apparatus 1A No. 398, with Leitz microscope stand, having an apochromatic condenser, was used for this work. All pictures were taken magnified 500 diameters.

The photographic method proved very convenient, for by means of it actual reproductions of spore material were made when the spores were at their best and the data were studied when convenient. This permitted a massing of data by means of which close comparative studies of microscopic characters could be made, which without photographs could not have been done.

Drawings, of course, might have been used instead, but there is no doubt that the photographic method is far superior to that of drawing. In the first place, actual reproductions of selected fields of spores are made instead of a few spores selected in accord with the personal preference of the worker and idealized and perfected in the process of drawing. Drawings are often misleading in their fine definitions and detail. Secondly, if the worker is careful to photograph an average field, which in most cases is easy to do, a great deal of additional data are recorded on the picture, which the one who was drawing would have to add in notes that take too much time when the critical stage of a large group of cultures is demanding his attention. Such data, for instance, are the percentage of spores with a certain number of septa, the percentage of macroconidia and microconidia, limits of size of different types of spores, etc.

Two complete sets of pictures were taken, except for cultures that would not fruit. One was of the fungi from cultures about 47 days old, grown on oat agar, and the other of fungi grown on lima bean agar when the cultures were about 10 days old.

The spores of some cultures would not take the stain; others, particularly those having mostly microconidia, even though perfectly sealed exhibited Brownian movement, and still other cultures produced so few spores that a field suitable to photograph was impossible to find. Many attempts, some of which were successful, were made to grow these refractory cultures on a medium that would produce a greater abundance of spores, and whenever successful pictures were taken. Notable

advance was made with some of them when grown on sterilized tomato stems and leaves.

In order to see what effect age and medium had on spore characters, three species, each typical of a group, were chosen and about 10 photographs were taken of them, grown on different media and at different ages. Our conclusions are indicated on page 354.

MICROSCOPIC NOTES

The only key published which includes the greater number of American species of Fusarium is Sherbakoff's (*18*), and it was therefore used as a basis for this work. In determining the various sections this key was very useful, but owing to the fine distinctions made between species and especially between varieties much difficulty was experienced in identifying an unknown culture.

In order to secure the necessary data for the identification work microscopical study was made of all cultures grown on hard oak agar and on lima bean agar, in addition to the data secured from our microphotographs of the cultures grown upon the same media, but under somewhat different conditions. The cultures grown on hard oat agar were about 15 weeks old and had been kept in the refrigerator at 10° C. The cultures grown on lima bean agar were about 4 weeks old and had been kept in a dark incubator at about 21° C.

As with the series of cultures used for the microphotographs, we had difficulty here also with certain ones not fruiting; and additional notes were made for certain cultures at about 4 weeks of age when grown on potato glucose agar and at various ages, depending upon the organism, when grown on tomato stems, potato plug, and lima bean agar.

In these examinations particular care was taken to include some mycelium in order to determine whether or not chlamydospores were present, for in the photographic work it was natural to select a field filled with spores rather than one filled with mycelium, and the presence or absence of chlamydospores, which character Sherbakoff used considerably, was often overlooked in the earlier studies.

MEDIA USED

A limited number of media was used in our work, for the concensus of opinion of those investigators who have used a large variety, notably Smith and Swingle (*19*), Apple and Wollenweber (*2*), and Sherbakoff (*18*), seems to be that little is gained from so doing. Sherbakoff believed that all important characters were brought out on hard oat agar, certain vegetable stems, tuber plugs, and potato agar containing about 5 per cent glucose. We followed the suggestion of Sherbakoff but used a few additional media. The formulas of the media follow:

OAT AGAR.—One hundred gm. rolled oats were put in 1,000 cc. of water and cooked for an hour in an Arnold steamer which varied in temperature from 50° to 75° C.

The product was strained through cheesecloth and the volume of liquid was brought up to 1,000 cc. Thirty gm. of agar were added, and the mixture was put in the autoclave and the pressure allowed to rise gradually to 15 pounds, where it remained for 15 minutes. The material was then tubed, and the tubes were plugged and autoclaved.

LIMA BEAN AGAR.—This was made in the same way as the oat agar except that the decoction was made by heating 100 gm. of broken pieces of lima beans in 1,000 cc. of water.

POTATO GLUCOSE AGAR.—This medium was made in the same way as the oat agar except that the decoction was made from 100 gm. of sliced potato tubers, and just before the mixture was tubed 50 gm. of glucose (T. J. Baker's c. p.) was added.

RICE.—About 3 gm. of rice were put into each tube, 10 cc. of tap water were added, and the tubes were plugged and autoclaved.

POTATO PLUG.—Cylinders cut from potato tubers were slanted and placed in tubes with enough water added to about cover the cylinder. The tubes were plugged and autoclaved.

SWEET CLOVER STEMS.—Stems of sweet clover (*Melilotus alba*) were cut into convenient pieces. If the stems were large enough only one was placed in a tube. Most of them, however, were small and two to four pieces were used. The stems were dry, so water enough to cover them was added. The tubes were plugged and autoclaved.

TOMATO STEMS.—Stems and leaves of young tomato plants (*Lycopersicum esculentum*) were cut into convenient pieces, put into tubes with distilled water added to within about ½ inch of the top of the stems. The tubes were plugged and autoclaved.

Five series of cultures were grown on oat agar, five on potato glucose agar, three on lima bean agar, two on potato tuber plug and one each on the other media mentioned above.

TEMPERATURE AND LIGHT CONDITIONS USED

By far the greater number of the series of cultures were grown in the dark in an incubator regulated between 20° and 22° C. This temperature, according to data of various investigations, seemed to be nearest the optimum for the greater number of species.

In all cases where the "dark" incubator is mentioned it refers to one the temperature of which was regulated by burning a 20-watt carbon light automatically controlled by a thermostat in the lower portion of the incubator. The cultures were kept in cans on wire screen shelves, and the light given off from this bulb should possibly be considered as influencing the results, though it does not seem probable that it did as the temperature of the room was such that the bulb was lighted but a small portion of the time.

One series of cultures grown on potato glucose agar was kept in diffused light and incubated at room temperature which varies from about 18° to 25° C.

Another series grown on potato glucose agar was inoculated in quadruplicate, and two tubes of each culture were grown in the "dark" incubator at 20° C. and two similar tubes grown in an incubator with glass doors, designated as the "light" incubator, which was in the greenhouse so placed that the cultures were in strong diffused light during the day. The tubes were kept in glass beakers and only a few in each beaker. The temperature of the "light" incubator was kept at 21.5° C.

Still another comparison between cultures grown in light and darkness was made with lima bean agar. In this case two tubes of each culture were kept in the dark incubator at 20° C. and two each were kept in diffused light in a room at a temperature which varied between 18° and 25°.

GENERAL DISCUSSION OF METHODS

EFFECT OF MEDIA

No distinct effort was made to determine the comparative values of different media, for in order to determine such values a large number of trials should be made on each medium under observation. However, the results which we gained from a few trials on a few media may be of some value.

EFFECT ON CULTURAL CHARACTERS.—The growth made on sweet clover stems lacked color. In fact certain cultures (those ordinarily grouped under K, a Discolor group, and under D, an Elegans group) which constantly produced color on all other media did not exhibit any on sweet clover. The growth was scanty in proportion to that on other media. This may have been due to the fact that the stems used were old ones which had been very dry. However, Sherbakoff (*18*) notes that the presence of the epidermis on stems seems to lessen the development of aerial mycelium and to favor production of fewer but better developed sporodochia. We also found that sweet clover stems seemed to favor the production of sporodochia and pseudopionnotes. A few cultures which had not at any time formed sporodochia did so on this medium. The sweet clover stems were sometimes covered with a thick, tough layer of plectenchymatic-like tissue which seemed never to bear spores.

The little experience we had with tomato stems suggested that they, too, favored the production of spores. However, our work with this medium was very limited, as we used it only on refractory cultures.

Lima bean agar proved to be another medium that did not stimulate color production. For instance on Group F (a Martiella group) notes taken 12 days after inoculation read, "a slight appearance of greenish blue growth," while cultures of the same group grown on oat at 11 days showed "various combinations of blues, greens, and purples." The D group (an Elegans group) which was mulberry purple on oat showed white or gray on lima bean.

That series of cultures (p. 350) growing on lima bean which was divided and part grown in the diffused light and part in the dark incubator showed practically no difference in color production. Only two cultures produced color in the dark that did not produce any in the light. The amount of mycelium on the lima bean cultures varied somewhat with the groups.

The mycelial growth on potato plug was abundant, and color appeared in varying degrees. That is, more color was produced on potato plugs than on lima bean or on the stems of sweet clover, but less than on the oat or potato glucose agars. Thick, tough layers of plectenchymatic tissue formed over the tuber plugs, as it did over the stems of sweet clover, but sporodochial growth was rare.

The greatest variety of colors was produced on rice, and finer group distinctions were brought out on this medium than on any other used in the cultural studies, but the colors were very mixed and they seemed not to stay true to group. Very little reliance was put on these results at the time the notes were taken. It is interesting to look back and see how nearly true to group the color determinations were. However, the mixture of colors produced was so difficult to describe that except for grouping purposes it is doubtful if rice as a cultural medium would have any specific value.

Again and again in the literature is noted the fact that for color production agar with glucose is the medium to use. Our results showed that the cultures grown on potato glucose agar did develop color, but not much more so than did those on oat agar. The color on the glucose agar in some cases was deeper than on the oat. For instance, notes taken at practically the same age on both media give for the color of group A (a Discolor group) on potato glucose "vinaceous cinnamon to orange cinnamon" and on oat it is "salmon buff to salmon color." For group C (a Discolor group) it reads "Bordeaux" on the glucose and "spinel pink" on the oat. However, the deepness of color is not constant throughout for special notes on culture No. 16 give "seashell pink" as the color on glucose and "cinnamon" as the color on oat. Our results would indicate that the two media are about equal in color production value and the amount of growth is practically the same, being abundant on both.

EFFECT ON MICROSCOPIC CHARACTERS.—No striking difference microscopically was noted between spores grown on lima bean, potato glucose, or oat agars (Pl. 1, A and B; 2, C). We found that the series of oat cultures kept in the refrigerator was in the best condition for spore study of all with which we worked, but it seems probable that this was due to the temperature rather than the culture medium, as the other oat series was about equal to the lima bean.

The few refractory cultures that were grown on tomato stems led us to believe that that medium might prove to be very good. At least it would be worth while to try it out further.

Appel and Wollenweber (*2*) concluded from their work that agar media were by no means so sure of producing normal conidia as the tubers, and the stems were found to be the most satisfactory of all. Wilcox, Link, and Pool (*20*) state that cultures grown on gelatin and agar media are not normal and can not be used in the determination of characters. Our results do not support these conclusions, but they confirm a statement made by Sherbakoff (*18*):

> An agar, especially such a one as oat hard agar, often gives all the forms of fructification for these fungi, with "normal" spores and more or less typical and brilliant color production.

It might be well to add here a footnote given by Sherbakoff (*18*) in explanation of the variance between his results and those of Appel and Wollenweber (*2*):

> This observation is apparently in some contradiction to the observations of Appel and Wollenweber (1910: 12–13), but indeed it is not so; because, judging by the "artificial" media actually used by them, their observations of unfitness of such media for study of "normal" growth of the Fusaria was based on "soft" agars too rich in sugar. The writer also found that such agars produced abnormal growth.

We found that oat agar more than any other medium used combined the qualities necessary to produce good cultural characters such as growth and color and normal spores.

EFFECT OF LIGHT

So little comparative work was done upon the effect of light on growth and color that our results are of rather limited value. The effect of light on Fusarium has never been thoroughly studied. Our conclusions and those of a few other investigators are included below.

Using lima bean agar for a medium, we found practically no difference in the amount of growth or the amount of color produced in cultures

grown in the dark incubator and those grown in diffused light, but the lima bean is not a good color producer under any condition.

With potato glucose agar there was some difference, though it was not striking. These comparisons were drawn, however, from cultures grown in the light incubator and others grown in the dark incubator (p. 350). Our notes taken when the cultures were a week old show that in the dark group A produced rufous pseudopionnotes; in the light they were ferruginous. A more intense purple color showed in group D when grown in the light than when grown in the dark. The most striking comparison, in fact practically the only striking one, was in the case of culture No. 69 which produced carmine mycelium in the light and white mycelium when grown in the dark.

Notes taken on a complete series of week-old cultures grown on potato glucose in diffused light showed practically the same results as those taken on the cultures grown in the dark incubator.

Smith and Swingle (*19*) found that often cultures which produced—

> a beautiful, rich salmon colored mycelium when grown in sunlight produced white mycelium when grown in a dark closet.

A difference in color was not noted on all media tried. Except for a difference in color these men concluded that light had no material effect on the growth.

Appel and Wollenweber (*2*) noted that conidia masses were much richer in color when grown in the light than in the dark. They also noted that when cultures were grown in the dark, poorly developed conidia with uneven septations and form appeared. Although direct sunlight was not exactly injurious the diffused daylight was most favorable in every way for the product of morphological characters.

EFFECT OF TEMPERATURE

We did no work with the effect of temperature on the fungi, but a summary of the conclusions of other workers may be of value.

In working with *Fusarium oxysporum*, Smith and Swingle (*19*) found that the fungus grows well on boiled potatoes at a temperature of from 15° to a little above 30° C. Below 15° the growth became slower and slower until 5° was reached, when practically no growth took place. Above 37½° no growth took place.

Link (*13*) did some detailed work on temperature relations of *Fusarium oxysporum* and *F. trichothecioides*. The optimum temperatures for the two are different. However, at temperatures between 15° and 20° C. a good growth was made by both.

Lewis (*11*) in his work with 24 cultures of Fusaria found that 20° to 25° C. seemed to be the best range of temperature for most of the cultures.

Appel and Wollenweber (*2*) in their summary of conditions which will guarantee a "normal growth" say that room temperature should be used, that is, "between 12° and 25° C. neither higher nor lower."

Edson and Shapovalov (*5*), working on temperature relations of six of the more common species of Fusarium, found that growth took place in varying amounts between 2° and 38° C. The minimum for growth was shown by *F. discolor* var. *sulphureum*, the maximum by *F. radioicola*. The maximum growth for all cultures took place between 25° and 30°, though growth was abundant between 15° and 30°.

A temperature of between 19° and 22° C. was used for practically all the work reported in this paper. Incidentally it was found that cultures kept in the refrigerator at 10° showed an unusually good spore condition. It was very likely due to the fact that so low a temperature inhibited the growth and was about the optimum temperature for preservation of spores after their formation. Cultures kept in the refrigerator were always kept at room temperature for about a week after they were inoculated.

During the later spring months the light incubator in the greenhouse warmed up in the middle of the day to 30° or 35° C. We found that cultures kept in this incubator during that time deteriorated quickly.

The conclusion drawn from our own experience and that of others was that cultures can be grown as well at room temperature as at a fixed temperature, provided the temperature does not go lower than 12° or higher than 25° C. In case it is desirable to keep spores in a normal condition for a longer period of time than is possible at room temperature this can be done by keeping them in a refrigerator at 10° or less.

EFFECT OF AGE

Careful study of the series of cultures grown for the purpose of noting the effect of age on spore formation was in some measure disappointing. Isolated examples could be found that would illustrate practically any theory one might wish to propound. Too many factors enter in, such as moisture, nutrients, temperature, etc., for one to be able to make definite conclusions as to the effect of age. It seems that if all conditions are right to produce a "hoch" [4] stage of a normal culture, age does not enter in more than that a very young culture or a very old one can not produce a "Hochkulture." Only relative age then, would seem to be of importance.

CONCLUSIONS AND SUGGESTIONS ON METHODS

The effects of media, temperature, light, and age, though not always very great on cultural and spore characters, are sufficient to make it advisable in describing characters to describe the culture media and conditions so freely as to make the repetition of the culture upon the same medium and under approximately the same conditions easily possible, to note under what conditions these results were observed, and to keep them within certain limits.

Our experience would suggest that cultures grown on a hard oat agar, in diffuse light, and at room temperature will give the best satisfaction.

[4] Appel and Wollenweber (2) in their attempt to find distinctive terms by which to designate the degree of development and the age of the cultures created six terms which are briefly defined as follows:

ANKULTURE: A little mycelium from the original substance of some Fusaria is inoculated on tubers or stems. A pure, rich mycelial culture results in which there are either no conidia or only a very few, and these are apt to be irregular in shape and septation and are not suitable for morphological research.

NORMKULTURE: A culture which produces conidia readily and in which the spores are regular in form and septation.

ABKULTURE: A culture in which deterioration has set in, and the spores which have not disintegrated are small and usually have fewer septations than do those in the Normculture. The Normkulture is subdivided into three stages:

The JUNGKULTURE, usually less than 8 days old, is one in which the spores have not reached a constant form of development, and spores of varying sizes and septations are found.

In the HOCHKULTURE the spores are truly normal, that is, comparatively even in size, shape, and septations.

In the ALTKULTURE the spores, due to lack of moisture or food, shrink a little in size; or if new spores are formed they are undersized, yet not deteriorated enough in form to belong to the Abkulture.

Plate 1, C and D, illustrate the "alt" and "hoch" stage of the Normkulture as seen in our work.

The cultures may be kept in a normal condition for 12 weeks or more by keeping them at a temperature of 10° C. or less.

Were the standardization work suggested on page 356 to be attempted, we would suggest as the media to be tested out a hard oat agar, potato stems, potato plugs, and possibly potato glucose agar. It would be advisable to try several regulated temperatures, together with a room temperature, the limits of which should be given, probably 12° to 25° C. Tests should be made to determine that time nearest which all the species reach the normal stage of their growth, and if possible some method should be devised to standardize moisture and humidity conditions.

PITFALLS IN IDENTIFICATION WORK WITH SPECIES OF FUSARIUM

The greatest obstacle in the way of accurate determination of species of Fusarium is the lack of a good monograph of the genus, and this lack is due in part to nonstandardization of the methods used in identification work, especially as regards kinds of media, environmental conditions, and the relative value ascribed to various characteristics of the fungus when grown in pure culture under laboratory conditions. The species and varieties intergrade and the differential characters used in the keys are not sufficiently distinct to permit any but an experienced investigator to use the key. To become an authority one must work long enough and with large enough numbers of species so that he can create within himself a conception of the species. In other words, he judges to what species the fungus in question belongs rather than actually identifying it.

Pathologists in various parts of the world often in connection with some pathological studies isolate a species of Fusarium. In their eagerness to name the organism which is causing economic loss they describe it so incomprehensively that future workers are not able to identify their cultures with it and therefore more new names appear.

Appel and Wollenweber (*2*) made a good beginning toward a monograph, but it was merely a beginning. Sherbakoff (*18*) has helped the situation somewhat with his "Fusaria of Potatoes," but his key is deserving of the criticism given above. He has split species up into so many varieties that to identify a specimen beyond its section becomes a tedious task of scientific guessing.

In the near future the botanical world, especially the mycological world, must determine and actively promote some policy with regard to trinomial nomenclature. If all the flowering plants were split into varieties on as many minor characters as are the fungi, binomial nomenclature would before long be a thing of the past. The American Code of Botanical Nomenclature (*1*) states in regard to categories of classification that the terms "subspecies" and "subgenus," etc., may be used when additional categories are necessary for the convenience in presentation of relationships, but "the term variety is relegated to horticultural usage."

The mycologist's difficulty arises largely from lack of perspective. The person who has worked on a single group for some time sees very real, fine distinctions which would not be at all significant to other mycologists. These distinctions may not be of enough importance to justify his making a new species, but they are too real to him to be overlooked, and he therefore originates a variety. Would it not be better to keep in mind that a species must have more or less flexible boundaries due to evolution which has taken and is now taking place,

and in describing new species or in identifying new cultures allow for a certain amount of variance? The code quoted just above defines species as "connected or coherent groups of individuals."

There is little doubt that in some cases physiological strains of species must be recognized by some system to be agreed upon, possibly by making new species, but until formally adopted by a representative body varietal names, especially those based on morphological characters, should be avoided.

SUGGESTION FOR A STANDARD METHOD FOR FUSARIUM STUDY

An important step in taxonomic work on Fusarium would be to standardize the methods for growing Fusarium species in somewhat the same way that certain bacteriological methods are standardized.

In order to do this it would first be necessary to carry on a comprehensive preliminary study. Interested workers in different localities would grow a large number of species of Fusarium, preferably subcultures from common stocks. The conditions of media composition, light, temperature, and humidity should be as nearly uniform as possible, selecting those suggested by the results of previous workers.

The method of note taking in the work should be sufficiently uniform to facilitate comparative studies of the results. From these comparative studies it could be concluded what conditions proved most satisfactory in growing species of Fusarium.

Selecting the most promising method thus obtained as a provisional standard, cultural work should again be carried on by a very large number of workers and with a very large number of species, and notes taken in a uniform manner. A comparison of the various notes on single species would determine whether or not the method used could be adopted as "a permanent standard."

By careful study of the various notes taken on all species of Fusarium used, the most stable characters could be determined and a really workable key made.

Such a procedure would involve a large expenditure of time and money and much care on the part of the workers. To find enough interested workers with the time to devote to such a study might in itself be a difficult task. However, until a key based on comprehensive data of this kind is made we see little hope for accuracy in identification. (See also page 354.)

RESULTS OF IDENTIFICATION WORK

The cultural and microscopic data, acquired as described above, were carefully studied and with the aid of Sherbakoff's (*18*) key many of the cultures under investigation were identified. Their descriptions and identifications follow according to groups. Practically all the cultures were found to be included under Wollenweber's three sections: Elegans, Discolor, and Martiella. Notable exceptions to this are No. 20, 69, and 75. For various reasons some few of the cultures are only provisionally identified, and two are not identified at all. The summary given in Table I will show which these are and give the reason for indecision. (See page 362.)

Since Sherbakoff's key was used, the descriptions of species given by him were taken as the standard in most cases. If questions arose about

individual characters, comparisons were made with descriptions by other investigators whenever such descriptions were available.

SECTION ELEGANS

One group (Group D) fell within this section. It included No. 21, 24, 25, 27, 28, 29, 31, 45, 46, 58, and 59. This was a difficult group to identify because of the scarcity of macrospores and the variations in color. Microscopically the group (with the exception of No. 59) falls into the species *Fusarium oxysporum*, but no tube culture produced sclerotia, which according to Smith and Swingle (*19*) were green in color and always found in cultures grown on potato plug, and according to Sherbakoff (*18*) were:

> Bluish black in color, constantly present on potato tuber plug and sometimes on different agars.

In plate cultures grown on potato agar with 5 per cent of glucose, a few of the numbers (21, 28, and 46) produced small, dark purple spots, which on examination proved to be masses of nonsporing mycelium; but after four weeks of growth these small masses of mycelium seemed to be too loose to be called sclerotia. The fact that they did not form consistently throughout the group also suggests that they are not the sclerotia noted by the authors mentioned above.

The color of our cultures, also, does not quite agree with former descriptions of the species. Sherbakoff gives "macroconidia in mass usually of pinkish buff color" but neglects to state on what medium this is true.

On potato-glucose agar plates kept in the light most of our cultures (No. 24, 25, 27, 29, 45, 58, and 59) showed salmon coloring varying from light buff to ochraceous salmon, but shades of purple are typically found. Combinations with pinks and sometimes with greens occur but a greater or less amount of purple was characteristic of the group under all conditions. The only media used in common with Smith and Swingle were boiled rice and potato tuber plugs. Smith and Swingle found the color on the former when grown in the dark "mixed pink and lilac shading into white." Our notes show a production of purple (true) to resolane purple. On potato tuber plug these authors noted the growth when made in the darkness was "pure white changing to creamy white." We noted a slight development of a pinkish and purple pigment when grown in the dark.

However, these discrepancies in color do not seem sufficient to throw these cultures out of *Fusarium oxysporum*, but the lack of sclerotia seems important We would, therefore, identify these cultures as *F. oxysporum* var. *asclerotium*,[5] a variety described by Sherbakoff which differs from *F. oxysporum*—

> by the absence of sclerotia, and definite plectenchymic sporodochia, in color of the mycelium and somewhat longer and narrower macroconidia.

Sherbakoff neglects to state in what way the color differs.

Macrospores were very scarce in all the cultures of this group, and in No. 24 none at all were found. In the other numbers they varied

[5] It might seem inconsistent after the discussion on binomial nomenclature on page 355 to make use of varietal names in our classification. We recognize the disadvantages of trinomial nomenclature but feel justified in following it to avoid still further confusion of the names used in this genus. We feel, however, that some official recommendations and action should be taken upon the important question by societies qualified to represent mycology.

considerably in size. This variation in size seemed to bear no consistent relationship to numbers, age, media, or temperature. We are therefore including in the limits given the various sizes of the spores measured on the different cultures.

Fusarium oxysporum Schlect. var. *asclerotium* Sherb. (Description taken from No 21, 24, 25, 27, 28, 29, 31, 45, 46, and 58.

Macroconidia typically dorsiventral, doesiventrality sometimes slight, usually distinct, and if so, ventrally curved. More or less uniform in diameter, with more or less abruptly attenuated apex; base pedicillate.

Microconidia very abundant. Oftentimes no macroconidia present, especially in cultures that have been kept for some time in stock. Chlamydospores common, mycelial intercalary and terminal, conidial intercalary.

Mycelium abundant, fine and long, from white to slight purple tint to haematoxylin violet to mulberry purple on hard oat agar grown in the dark; white to slight development of pinkish pigment and sometimes purple color on potato plug grown in dark; white to slight purple tint on potato-glucose agar grown in darkness and white to cameo pink to petunia violet when grown in the light.

Substratum colorless to purple to dull purplish black on potato-glucose agar.

The conidial measurements are as follows:

1-septate, few, 22.5 by 4.5 microns.

2-septate, rare, none measured.

3-septate, 50 to 100 per cent, 34 by 4 (22 to 52 by 3 to 5 microns).

4-septate, 0 to 40 per cent, 48 by 4.5 (35 to 60 by 4 to 5 microns).

5-septate, 0 to 20 per cent, 48 by 4.5 (35 to 60 by 4 to 5 microns).

Fusarium sclerotioides Sherb. var. *brevius* Sherb. (Description from No. 59.) See Plate 2, A.

Macroconidia typically dorsiventral; dorsiventrality distinct, ventrally curved, dorsally elliptic, typically broader toward apex, indistinctly pedicillate; gradually attenuated, pointed apex.

No sclerotia, no plectenchymic sporodochia.

Microconidia abundant, oval, 0-and 1-septate.

Sometimes far in excess of macroconidia.

Intercalary mycelial chlamydospores common. Mycelium well developed, white to mulberry purple on hard oat agar; white to cameo pink and petunia violet when grown in the light on potato glucose agar; slight development of pinkish pigment on potato plug. Substratum on potato glucose agar livid pink to dark maroon purple.

The conidia measurements are as follows:

1-septate rare, 22 by 4 microns.

2-septate rare, no measurements made.

3-septate 50 to 80 per cent, 36 by 5 (25 to 40 by 4.5 to 5.5) microns.

SECTION DISCOLOR

Three distinct groups, namely A, B, K, and one group C, which varied considerably within itself, contained species included in Wollenweber's section Discolor. The description of these cultures with their identification follows.

Fusarium trichothecioides Wr.; (Description taken from No. 1, 3, 5, 6, 7, 8, 9, 10, 11, 12, 14, 33, 38, 61, 63, 64, 76, 79, 81, 83, 91, and 97.)

Conidia not differentiated into macroconidia and microconidia, but there are two types of spores, the comma and the discolor types. The discolor type, that is conidia shaped like those of *Fusarium discolor*, is very rare. Comma type of spore slightly dorsiventral to straight, diameter more or less uniform, apex and base not differentiated rounded. (Pl. 2, B.)

Spores in powdery masses, at first on aerial mycelium, which soon covers the surface completely.

Terminal and intercalary chlamydospores occasionally noted. Mycelium abundant at first and white, soon becoming covered with powdery spore masses, which vary from pale flesh, salmon buff, chamois to buckthorn brown on potato-glucose agar; pale flesh to pale pink to pale pinkish buff on potato plug; white to pale flesh to safrano pink to light ochre to salmon buff on oat agar.

Substratum colorless to somewhat darkened on potato-glucose agar. This species is easily identified by its very characteristic powdery growth.

The conidial measurements of the comma type of spore are as follows:
0-septate, 11 by 4 (6 to 19 by 3.5 to 4.5) microns.
1-septate, 15 by 4 (12 to 24 by 3.5 to 5) microns.
2-septate, 20 by 4.5 (18 to 26 by 4 to 5) microns.
3-septate, 26 by 5 (19 to 34 by 4.5 to 6) microns.
4-septate, 32 by 5 microns.

The percentages of 0-, 1-, 2-, and 3- septate conidia vary in different cultures. In most cases 1-septate conidia predominate.

Fusarium subpallidum var. *roseum* Sherb. (Description taken from No. 15, 17,[6] 22, and 40.)

Macroconidia typically dorsiventral, dorsiventrality distinct, ventrally curved, more or less uniform diameter, apex not long, rounded; base pedicillate.

Spores in small salmon-colored sporodochia, occasionally merging into pseudopionnotes. Microconidia absent. Chalmydospores sometimes found, both conidial and mycelial intercalary. Mycelium somewhat varied in color, white above to salmon orange below with some shades of purple on potato plug; flesh pink to light coral pink and rose pink with occasional slight tint of purple or yellow on potato agar with 5 per cent glucose.

Substratum, colorless to rose pink on potato agar with glucose.

The conidial measurements are as follows:
1-septate, 0 to 20 per cent, 18 by 4 microns.
2-septate, rare (no measurements made).
3-septate, 50 to 90 per cent, 25 by 4.5 (16 to 30 by 3.5 to 6) microns.
4-septate, 0 to 15 per cent, 34 by 5 microns.
5-septate, 0 to 12 per cent, 34 by 5 microns.

It is doubtful whether there is enough difference between the species *Fusarium subpallidum*, *F. subpallidum* var. *roseum*, *F. clavatum*, and *F. discolor* to warrant more than one species.

Fusarium clavatum Sherb. (Description taken from No. 23, 41, 42, and 43.)

Macroconidia typically dorsiventral, dorsiventrality distinct, ventrally curved, slightly broader toward the apex, apex rather abruptly attenuated, base distinctly pedicillate.

Spores in small sporodochia, later merging into pseudopionnotes from pale flesh to salmon colored on oat and lima bean; light coral red to coral red on potato agar with 5 per cent glucose.

Microconidia absent.

Intercalary conidial chlamydospores sometimes present, mycelial intercalary chlamydospores occasionally found or scattered.

These cultures showed unusually close identity culturally and microscopically throughout.

The conidial measurements are as follows:
1-septate, rare.
2-septate, 2 to 6 per cent.
3-septate, 75 to 90 per cent, 27 by 4.5 (16 to 40 by 4 to 6) microns. Average limits 20 to 30 by 4 to 5 microns.
4-septate, 15 to 30 per cent, 30 by 5 microns.
5-septate, 5 to 15 per cent, 30 by 5 microns.

Practically speaking, the spore measurements and the percentages of the variously septated spores varied no more between the different media (potato glucose, oat, and lima bean agars) than between different cultures grown on the same medium.

No. 16 seems to vary between *F. clavatum* and *F. subpallidum* var. *roseum* and *F. discolor* in color characters, but the spores agree with *F. discolor* in shape and size, and sometimes in average number septations. We, therefore, are identifying it as that species.

[6] Practically no normal spores were found in any of the cultures of No. 17 on any of the media used. The culture seems attenuated. However, from the few spores and from earlier cultural notes we identified it with this species.

Fusarium discolor Ap. and Wr. var. *sulphurum* (Schlect) Ap. and Wr. (Description taken from No. 77 and 85.)

Macroconidia typically dorsiventral, dorsiventrality distinct, ventrally curved, more or less uniform in diameter. Apex not long, typically slightly broader toward the apex, more or less abruptly attenuated, base distinctly pedicillate.

Spores in pseudopionnotes, flesh ochre to salmon color on all media used.

Microconidia absent.

Conidial chlamydospores often found; mycelial chlamydospores never noted, due, perhaps, to scarcity of mycelium.

Mycelium white at first, but soon becoming entirely covered by pseudopionnotes.

Substratum colorless to slight salmon coloring.[7]

(For photograph of No. 77, see Pl. 1, C and D.)

The conidial measurements are as follows:

2-septate, rare.

3-septate, 15 per cent, 24 by 4 (22 to 32 by 4 to 4.5) microns.

4-septate, 15 per cent, 35 by 5 microns.

5-septate, 70 per cent, 40 by 5 (35 to 45 by 4 to 5) microns.

6-septate, rare, 42 by 6 microns.

The percentages of the different septate spores vary in different cultures. For instance, 5-septate spores were 97 per cent on 7-day culture on lima bean and only 40 per cent on oat about 15 weeks old. The size also varies. The cause of difference in size would seem to depend on temperature and moisture conditions quite as much as on the medium used.

No. 84 was much the same as No. 77 and 85 but showed the following variations in spore measurements:

1-septate, rare.

2-septate, 4 per cent.

3-septate, 40 per cent, 26 by 4.5 microns.

4-septate, 30 per cent, 32 by 4.5 microns.

5-septate, 25 per cent, 36 by 5 microns.

6-septate, rare, 38 by 5 microns.

Color of growth lighter on potato glucose, orange tinge with bacterial-like growth below.

No. 52 was much the same as No. 77 and 85 but showed the following variations in spore measurements:

3-septate, 12 per cent, 25 to 40 by 4.5 microns.

4-septate, 8 per cent, 38 by 4.6 microns.

5-septate, 80 per cent, 42 by 5 (35 to 52 by 5) microns.

6-septate, rare.

Spores seem to be slightly less curved than in No. 77, though they grade into each other.

Color of growth slightly different, apricot orange rather than flesh ocre on all media used. On lima bean mycelium was medium in growth, contrasted with its scarcity in No. 77.

The darkening of the medium mentioned in note on *Fusarium discolor* var. *sulphureum* was never noticed in cultures of this number.

F. culmorum (W. Smith) Sacc. (Description taken from No. 84, which was the only isolation made of this species.) See Plate 2, D.

Macroconidia dorsiventral, ventrally straight or very slightly curved, slight constriction at the apical end and the pedicillate base, quite uniform diameter throughout, typically 5-septate 36 by 6 microns. 3- and 4-septate conidia are not uncommon. Conidia have thick membranes and very pronounced septa. Orange-colored sporodochia found.

Conidial chlamy dospores abundant on lima bean agar, age 175 days.

On potato glucose agar mycelium abundant, bright pink above, carmine to ox-blood red below.

Substratum ox-blood red.

SECTION MARTIELLA

The members of the one group (F) that fell within this section were not identical in their cultural characters but were sufficiently similar to suggest a group. Microscopically it is quite easy to recognize the group

[7] On potato glucose agar, both in the light and in the dark, the medium sometimes darkened to a brown black and the growth became more or less powdery, from Sanford's brown to a nigger brown in color, the pseudopionnotes disappearing. Spores mounted from such cultures appeared more or less disintegrated.

Martiella, for the blunt spores are very characteristic. The size of the spores and number of septations vary considerably, and to identify the species and varieties offers many difficulties.

Sherbakoff (*18*), in this group also, has made varieties that could have been avoided had he made his species a little more comprehensive, and from our experience the characters of some of his species are not sufficiently stable. Such characters are comparative width and length of spores, "somewhat narrower macroconidia," "color of conidia and substratum usually paler," "frequent occurrence of bluish plectenchyma," etc.

After considerable comparative study we have identified No. 30, 35, 36, 47, 49, 51, 53, 71, 74, 80, 89, 90, 94, and 95 as *Fusarium solani*. Slight differences occur in these cultures, which, judging from a single photograph or a single set of notes, might suggest a variety of *F. solani* or of *F. Martii* or even a new species, but study of all of the data shows that these cultures do not have sufficiently stable characters to identify them as varieties or species. The cultures vary from one another and from the descriptions of *F. solani* only in minor details. Some of these variations are shown in the two photographs of *F. solani*. (Pl. 3, A and B.)

Fusarium solani (Mart. p. par.) Ap. et. Wr. (Description taken from No. 95 as a type.)
Macroconidia typically somewhat broader in upper half, rounded to slightly constricted apex, slightly if at all pedicillate. Normally 3-septate 28.75 by 4.5 microns (limits 27 to 38.5 by 4 to 5 microns), sometimes 2- and 4-septate, rarely 5-septate.
Pseudopionnotes and sporodochia occur commonly on most media.
Microconidia may or may not be present. When present usually abundant, round or oval in shape. Chlamydospores in mycelium terminal and intercalary, common in old cultures.
Aerial mycelium weak to well developed, typically white, neutral gray, sometimes with a purple tint.
Substratum on potato glucose agar usually from deep purplish vinaceous to dull violet black. Color on oat agar a mixture of blue, green, and purple.

Fusarium coeruleum (Lib.) Sacc. (Description taken from No. 55, which was the only isolation made of this species.)
Macroconidia dorsiventral, slightly ventrally curved. Basal end distinctly pedicillate. Apex rounded, more or less abruptly attenuated. Uniform diameter throughout.
Three-septate spores dominant, quite variable, 31 to 42 microns by 5 to 6 microns.
Aerial mycelium feltlike in age, appressed, white to bluish white and olive buff to dusky slate, violet on potato glucose agar.
Plectenchymatic tissue and substratum on potato glucose agar violet to indigo blue and bluish black.
Chlamydospores very abundant in old cultures, terminal and intercalary and in long chains and masses.

OTHER SECTIONS AND UNIDENTIFIED ISOLATIONS

The few cultures that fell outside of the three sections just discussed were identified by means of Sherbakoff's key and descriptions (*18*), but since we did not have known cultures for comparison, no descriptions of them are included here. The identification of each as we determined it is as follows:

Section Gibbosum: No. 20, *Fusarium gibbosum*.
Section Roseum: No. 13, *F. subulatum* var. *brevius*.
Section Arthrosporiella: No. 69, *F. arthrosporioides;* No. 72, *F. anguioides*? (Chlamydospores were sometimes found.)
Section Ferruginosum: No. 75, *F. bullatum* (may be variety *roseum*).

TABLE I.—*Summary of identifications made, together with source of diseased tubers, character of disease and date of isolation*

Isolation No.	Source of tubers.	Date of isolation.	Suspected disease.	Determination.
1	Roundup	Feb. 20, 1914	Dryrot	*F. trichothecioides.*
3	do	do	do	Do.
5	do	do	do	Do.
6	do	do	do	Do.
7	Stevensville	do	do	Do.
8	do	do	do	Do.
9	do	do	do	Do.
10	do	do	do	Do.
11	Lavina	Mar. 15, 1914	do	Do.
12	do	do	do	Do.
13	Miles City	Apr. 1, 1914	do	*F. subulatum* var. *brevius.*
14	do	do	do	*F. trichothecioides.*
15	Moccasin	Jan. 15, 1915	do	*F. subpallidum* var. *roseum.*
16	do	do	do	*F. discolor.*
17	do	do	do	*F. subpallidum* var. *roseum.*
20	do	do	do	*F. gibbosum.*
21	do	do	do	*F. oxysporum* var. *sclerotium.*[1]
22	do	do	do	*F. subpallidum* var. *roseum.*
23	do	do	do	*F. clavatum.*
24	Rockvale	do	Wilt	*F. oxysporum* var. *sclerotium.*[1]
25	do	Jan. 16, 1915	do	Do.[1]
27	do	do	do	Do.[1]
28	do	do	do	Do.[1]
29	do	do	do	Do.[1]
30	do	do	do	*F. solani.*
31	do	do	do	*F. oxysporum* var. *sclerotium.*[1]
33	Great Falls	Jan. 15, 1915	Dryrot	*F. trichothecioides.*
35	Rockvale	Jan. 21, 1915	Wilt	*F. solani.*
36	do	do	do	Do.
38	Fallon	Jan. 22, 1915	do	*F. trichothecioides.*
40	Moccasin	Jan. 26, 1915	do	*F. subpallidum* var. *roseum.*
41	do	do	do	*F. clavatum.*
42	do	do	do	Do.
43	do	do	do	Do.
45	Corvallis	Feb. 3, 1915	do	*F. oxysporum* var. *sclerotium.*[1]
46	do	do	do	Do.[1]
47	do	do	do	*F. cF. solani.*
48	do	do	do	*ulmorum.*
49	do	do	do	*F. solani.*
51	Bozeman	June 5, 1915	Dryrot	Do.
52	Wibaux	do	do	*F. discolor* var. *sulphureum.*
53	Bozeman	June 7, 1915	do	*F. solani.*
55	do	June 22, 1915	do	*F. coeruleum.*
58	Lewistown[2]	Aug. 23, 1915	Wilt	*F. oxysporum* var. *sclerotium.*[1]
59	do	do	do	*F. sclerotioides* var. *brevius.*[1]
61	Great Falls	Sept. 7, 1915	do	*F. trichothecioides.*
63	Havre	Dec. 10, 1915	Dryrot	Do.
64	Roundup	Mar. 21, 1916	do	Do.
69	Billings	Apr. 20, 1916	do	*F. arthrosporioides.*
71	Darby	May 4, 1916	do	*F. solani.*

[1] See notes on Elegans group p. 357.

[2] There is some confusion in the record of the source of this culture. It is recorded in one place as Big Arm and in another as Lewistown. Circumstantial evidence points to the latter as correct.

TABLE I.—*Summary of identifications made, together with source of diseased tubers, character of disease and date of isolation*—Continued

Isolation No.	Source of tubers.	Date of isolation.	Suspected disease.	Determination.
72	Bozeman	May 1, 1916	Dryrot	*F. anquioides.*
74	Havre	June 27, 1916	do	*F. solani.*
75	Forsyth	Feb. 1, 1917	do	*F. bullatum.*
76	do	May 22, 1917	do	*F. trichothecioides.*
77	do	do	do	*F. discolor* var. *sulphureum.*
79	Stone Shack	do	do	*F. trichothecioides.*
80	Bozeman	do	do	*F. solani.*
81	Glendive	do	do	*F. trichothecioides.*
83	Billings	do	do	Do.
84	do	do	do	*F. discolor* var. *sulphureum.*
85	do	do	do	Do.
89	Glasgow	June 12, 1917	do	*F. solani.*
90	Boulder	do	do	Do.
91	Hardin	do	do	*F. trichothecioides.*
94	Joliet	June 14, 1917	do	*F. solani.*
95	Bozeman	June 16, 1917	do	Do.
97	Circle	Mar. 23, 1918	do	*F. trichothecioides.*

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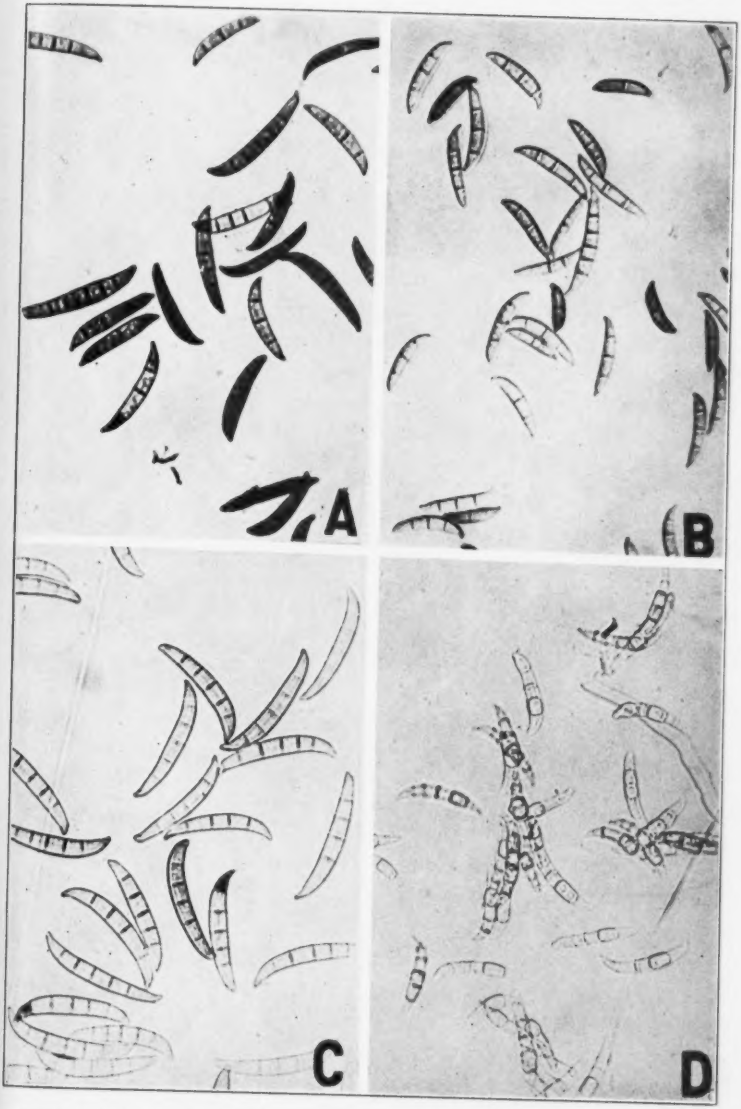
PLATE 1

A.—*Fusarium clavatum*, No. 41, grown on potato glucose agar. Age 43 days.
B.—*Fusarium clavatum*, No. 41, grown on oat agar. Age 47 days.
C.—*Fusarium discolor* var. *sulphureum*, No. 77, grown on lima bean agar. Age 7 days. (Hochkulture.)
D.—*Fusarium discolor* var. *sulphureum*, No. 77, grown on lima bean agar. Age 91 days. (Altkulture.)


A
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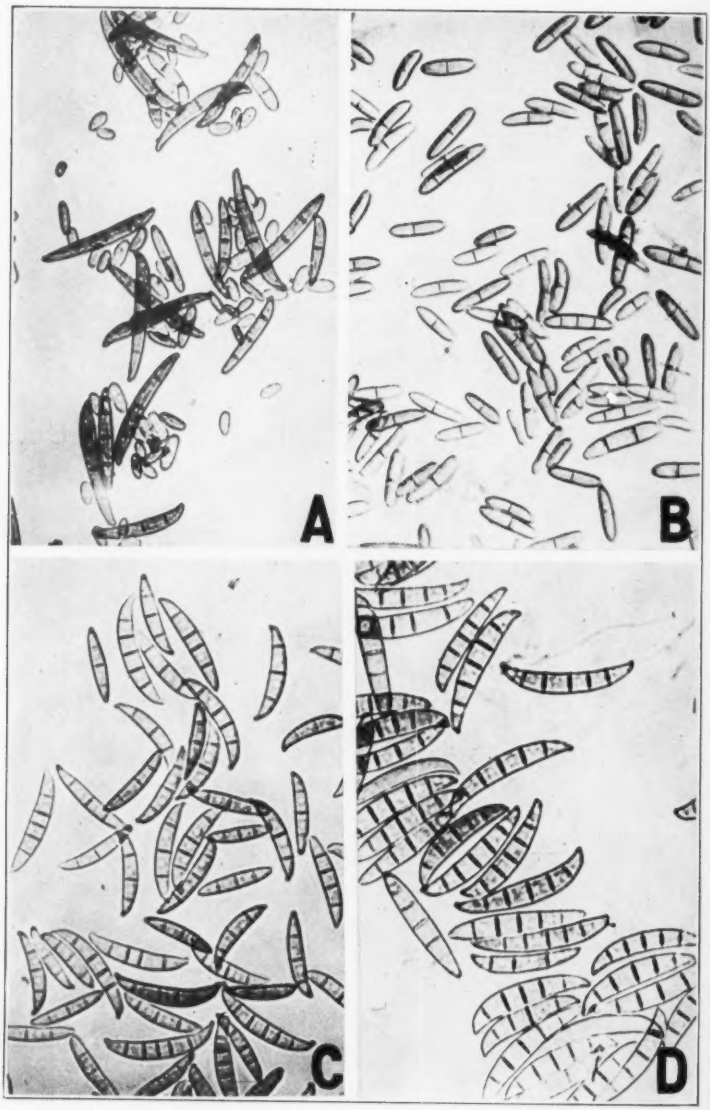
A
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D

PLATE 2

A.—*Fusarium sclerotioides* var. *brevius*, No. 59, grown on lima bean agar. Age 9 days.
B.—*Fusarium trichothecioides*, No. 38, grown on oat agar. Age 45 days.
C.—*Fusarium clavatum*, No. 41, grown on lima bean agar. Age 12 days.
D.—*Fusarium culmorum*, No. 48, grown on lima bean agar. Age 10 days.

PLATE 3

A.—*Fusarium solani*, No. 95, grown on tomato leaves and stems. Age 37 days.
B.—*Fusarium solani*, No. 47, grown on tomato leaves and stems. Age 34 days.

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Species of Fusarium Isolated from Potato Tubers
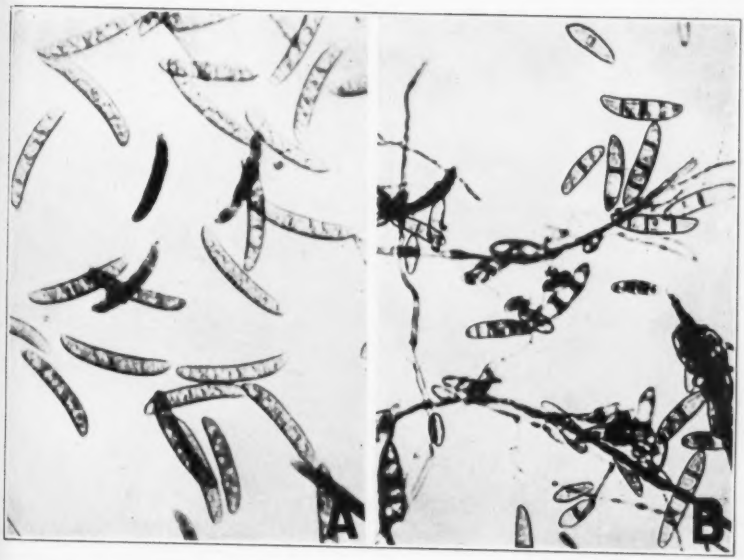
PLATE 3